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# The Yale Journal of Biology & Medicine


301 [illegible] *F. Duran-Reynals*

313 [illegible] *W. Lane Williams and Richard B. Aronsohn*

333 [illegible] *Alfred Gottschalk*

353 [illegible] *V. O. Hurme and G. VanWagenen*

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# Kodak reports to laboratories on:

**making one's point vividly in a learned journal . . . a reagent for determining α-keto acids in blood and urine . . . riding the new speed vs. sharpness curve**

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ERRATUM

The Editors regret the omission of the plus and minus signs between the individual values and their standard deviations in Tables 1 and 2 of the paper by Knapp, Hyman and Beitel entitled "A method for the estimation of regional cerebral blood flow" in the December–February number, pp. 365 and 366. This omission has been corrected in the reprints which are available from the authors.

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# Teamwork in research

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F. DURAN-REYNALS*

*Department of Microbiology, Yale University School of Medicine*

# ON THE PARALLEL BEHAVIOR OF CANCER AND BACTERIAL CELLS IN THE SAME HOST: EXPERIMENTS WITH CORTISONE AND A TRANSPLANTABLE MOUSE TUMOR†

Of the numerous and important effects of cortisone recognized thus far, this paper is primarily concerned with two. One is the inhibition of a primary tumor growth with the concurrent promotion of metastases; the second is the initiation of infection by saprophytic bacteria latent in tissues. The present study has been designed to observe the simultaneous occurrence of these two phenomena in the *same* host so that they may be closely compared.

## MATERIALS AND METHODS

*The HAS sarcoma.* The tumor used in the present study is a mouse transplantable tumor growing in its strain of origin. It is a sarcoma which has been perpetuated in this laboratory since 1947 and was initially induced by the inoculation of 1 mg. of methylcholanthrene, dissolved in 0.1 ml. of sesame oil, into the peritoneal cavity of a C57 black mouse 4 months old. The mouse succumbed 130 days after inoculation bearing a growth 2.0 x 1.5 cm., which had extended to involve the pleura, diaphragm, and liver. Grossly, the tumor was soft and somewhat viscid; microscopically, it proved to be a sarcoma comprised of bundles of large fibroblasts with clear cytoplasm and frequent mitoses. The sarcoma grows readily when transplanted to C57 black mice, the percentage of successful takes being 100% in most passages. Such tumor-bearing mice live 6 or more weeks after grafting.

Since its induction, the HAS tumor has been maintained by monthly cell transplants to C57 black mice, and it is now approximately in its 100th passage. When harvested and transplanted, the tumors usually average 3 cm. in diameter, are frequently necrotic at the core, but never induce metastases or inflammatory lesions in the viscera. Throughout successive passages the sarcoma has maintained the microscopic features of the original tumor although the amount of intercellular material has, in general, increased. This histological feature, in frequent association with areas of necrosis, has given the tumor a characteristic appearance (Figs. 1, 2, 3, 5, 7).

In transplanting the tumor, a mouse bearing a sizable growth is sacrificed and neoplastic tissue is immediately excised and dissected free from grossly necrotic mate-

* Lecturer and Research Associate in Microbiology.

† This investigation was aided by research grants from The Jane Coffin Childs Memorial Fund for Medical Research, the National Cancer Institute of the National Institutes of Health, United States Public Health Service [USPHS C997 (C6)], and the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council.

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rial. The tumor tissue is transferred to a glass homogenizer and ground with 20 volumes of saline. A 0.1 ml. inoculum of the cell suspension is injected into the groin of susceptible animals, usually C57 black mice, ranging in age from 6-8 weeks. In several experiments, 3,000 units of penicillin and 4 mg. of streptomycin were added to 10 ml. of the cell suspension 1 hour before injection. This either reduced considerably or entirely suppressed the number of contaminating organisms. In general, tumor growth was apparent 1-2 weeks after inoculation.

*Cortisone.* The hormone has been kindly supplied by Merck & Company (Cortone Acetate). The mice received subcutaneous injections of 0.5 mg. per day during the 3 days prior to tumor inoculation, and then 0.1 mg. daily (except week-ends) until the mouse died or was sacrificed. During the average span of 2 months after inoculation a mouse would receive a total of 7 or 8 mg. of cortisone. No important changes in body weight were observed.

*Lyophilized tumor tissue.* The tumor suspension, extracted with 1 or 2 volumes of water, was lyophilized in the usual way. Each mouse was given 3 intraperitoneal injections each of 15 mg. of the dried material; the first inoculation was given on the day the tumor was injected and the two subsequent doses at 5-day intervals. The effects of lyophilized tumor tissue in heterologous strains of mice is discussed by Molomut, *et al.*[38] and Kahss, *et al.*[39]

## EXPERIMENTAL

*The properties of the HAS tumor.* Apart from the gross and microscopic characteristics of the HAS tumor which have persisted through 100 passages in C57 black mice, certain strain specificities have been noted. For example, although the tumor grows well in C57 leaden mice, where our data reveal 60 growths out of 60 tests, it could be transplanted to only 4 of 12 C57 brown mice. Curiously, the tumor grew until the death of the animal in 4 hairless mice. On the other hand, regression followed short periods of growth in Swiss, A, C3H and DBA mice. All these data refer to mice inoculated subcutaneously with 0.1 ml. of a 1 : 20 cell suspension. If much larger quantities were used, for example, if 0.5 ml. of a 1 : 5 cell suspension were injected into Swiss mice, then the tumor grew as well as it did in the C57 black strain.

A striking characteristic of the tumor is its pronounced viscidity, best shown when the tissue is ground and extracted. This viscidity disappeared promptly upon addition of preparations of bull testis or streptococcus rich in hyaluronidase,* indicating the presence of abundant hyaluronic acid and/or other polysaccharides. This finding was confirmed by the late Dr. Henry Bunting, who demonstrated a strong toluidine blue metachromatic reaction in the tumor which disappeared after treatment with the enzyme preparation.† From the abundance and nature of its intercellular matrix,

* Supplied by Wyeth Institute of Applied Biochemistry.

† These studies, by Dr. Bunting, are briefly mentioned on page 981 of his paper.[7]

the HAS tumor belongs in the same category as certain other tumors such as some of the avian sarcomas,[3, 22] human endotheliomas[24] and neoplasms of synovial tissue.[25]

**TUMOR PASSAGE IN CORTISONE-TREATED MICE**

These studies concerned the transplanting of the HAS tumor to C57 black mice and may be grouped according to treatment:

A. *Cortisone-treated mice.* 17 tumor passages in 14 months. In the first 5 passages *lyophilized* tumor tissue was injected in addition to the cell suspension.

B. 1. *Cortisone-treated mice.* 5 tumor passages in 2½ months.

2. *Cortisone-treated mice.* 4 tumor passages in 3 months with antibiotic added to the inoculum.

C. *Untreated mice.*

The growth of all tumors was recorded by weekly measurements. Since the tumor passages in the various groups of this series were undertaken at approximately the same intervals, often on the same day, the results may be compared directly.

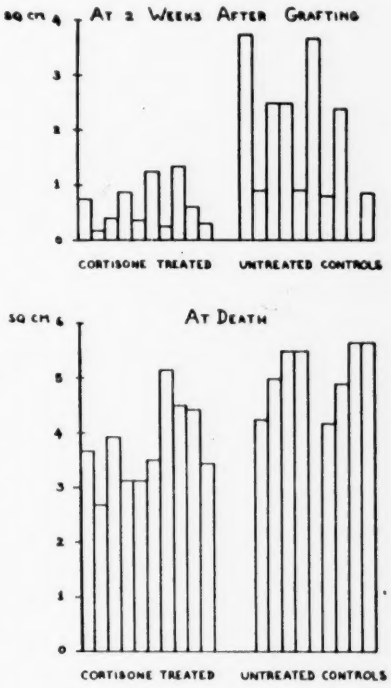



FIG. A. Effect of passages of the HAS tumor through mice treated with cortisone. Each column represents the average size of the primary tumor in the mice of the 13th-17th passage of Experiment A and of the five passages of Experiment B2.

*Effect of cortisone on local tumor growth.* From the data available in the last 5 passages of group A and the 5 passages of B2 (Table 1, Fig. A), it is evident that cortisone has a marked inhibitory effect on the initial growth of the primary tumor, an effect which is considerably diminished at the death of the animals. No accurate information is available from the first 5 passages of Experiment A and the 4 passages of Experiment B1, and tumor inhibition, if present, was not produced. It may well be that the overwhelming infection present during these passages interfered with the corti-

TABLE I. INHIBITION OF THE PRIMARY GROWTH IN MICE GRAFTED WITH THE HAS SARCOMA AND TREATED WITH CORTISONE

| | | *Two weeks after grafting* | | *At death* | | |
|---|---|---|---|---|---|---|
| | *Passage* | *No. mice* | *Average size tumors* | *No. mice** | *Average age days* | *Average size tumors* |
| | | | *cm.* | | | |
| Exp. A | Treated 13 | 6 | 0.95 x 0.75 | 5 | 40 | 1.75 x 2 |
| | Control | 6 | 2.16 x 2.0 | | All mice killed at 16th day | ..... |
| | Treated 14 | 8 | 0.45 x 0.40 | 7 | 46 | 1.44 x 1.70 |
| | Control | 10 | 1.00 x 0.85 | 3 | 40 | 2.06 x 2.12 |
| | Treated 15 | 4 | 0.62 x 0.62 | 3 | 43 | 2.00 x 1.91 |
| | Control | 10 | 1.68 x 1.66 | 5 | 38 | 2.25 x 2.25 |
| | Treated 16 | 5 | 1.05 x 0.9 | 4 | 47 | 1.68 x 1.75 |
| | Control | 10 | 1.68 x 1.66 | 2 | 48 | 2.62 x 2.25 |
| | Treated 17 | 6 | 0.62 x 0.54 | 6 | 45 | 1.79 x 1.66 |
| | Control | 10 | 1.02 x 0.95 | 4 | 40 | 2.45 x 2.37 |
| Exp. B2 | Treated 1 | 6 | 1.33 x 0.95 | 3 | 45 | 1.87 x 1.87 |
| | Control | 6 | 2.16 x 2.00 | 6 | All mice killed at 16th day | ..... |
| | Treated 2 | 7 | 0.50 x 0.50 | 5 | 45 | 2.43 x 2.15 |
| | Control | 10 | 1.00 x 0.85 | 4 | 50 | 2.06 x 2.12 |
| | Treated 3 | 5 | 1.20 x 1.20 | 4 | 45 | 2.20 x 2.12 |
| | Control | 10 | 1.68 x 1.66 | 5 | 35 | 2.25 x 2.25 |
| | Treated 4 | 5 | 0.75 x 0.85 | 4 | 48 | 2.06 x 2.18 |
| | Control | 10 | not measured | 2 | 48 | 2.62 x 2.25 |
| | Treated 5 | 6 | 0.45 x 0.50 | 5 | 42 | 1.90 x 1.65 |
| | Control | 10 | 1.02 x 0.95 | 4 | 45 | 2.45 x 2.37 |

* The number of mice was somewhat reduced due to natural or intentional early death.

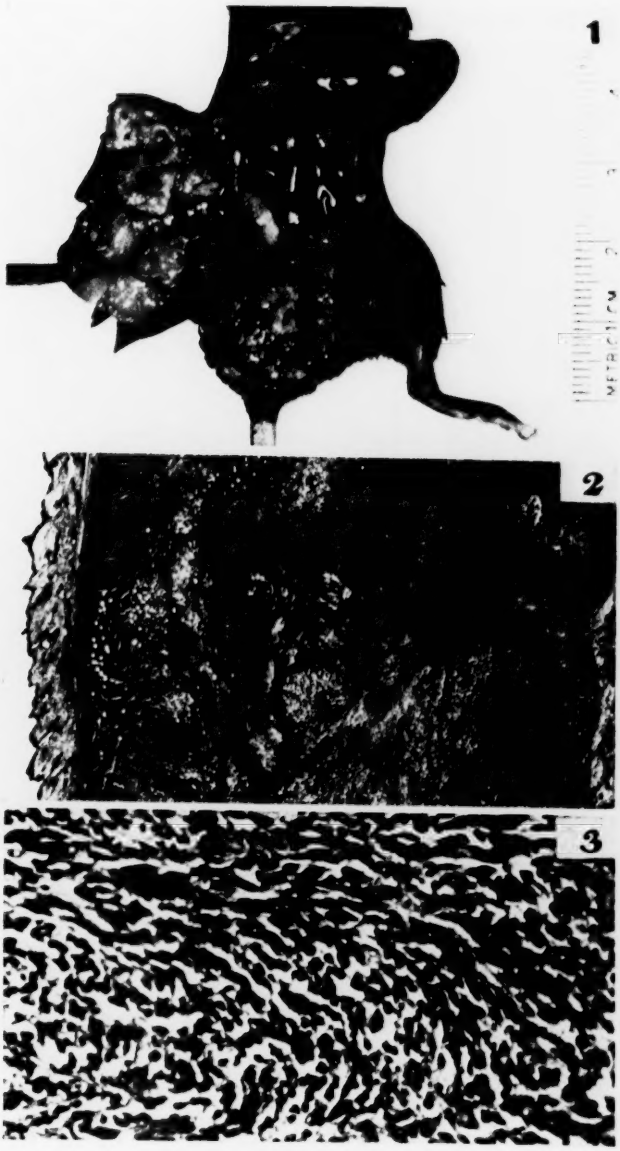
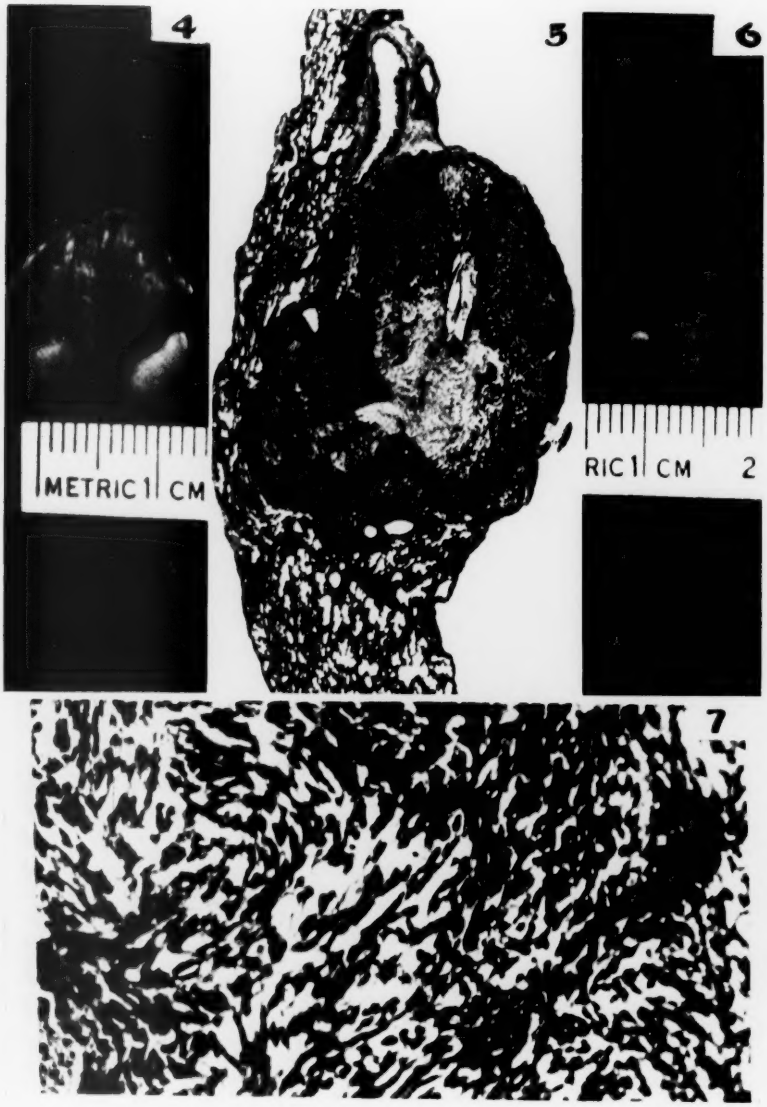



FIG. 1. The HAS tumor at its 60th passage, 6 weeks after grafting. Note typical pattern and absence of inflammation in viscera.

FIGS. 2 and 3. The tumor mag. ×20 and ×90 respectively.




FIGS. 4 AND 6. Lung metastases of the HAS tumor in the cortisone-treated mice. Note inflammatory lesions in the latter.

FIGS. 5 AND 7. Lung metastases as above, magn. ×24 and 300, respectively.

FIG. 8. Liver of a tumor-bearing cortisone-treated mouse showing typical gross inflammatory lesions.

FIG. 9. Liver as in Figure 8 (magn. ×10).

FIG. 10. Spleen of a similar mouse (magn. ×20).

some effect, a phenomenon which has been reported for tissue repair.[20] When primary tumor inhibition was quite apparent it was difficult to say whether the hormone promoted additional necrosis in the tumor. If it did, the effect was not remarkable.

*Effect on the development of metastases and inflammatory lesions.* From the data presented in Table 2 it is evident that lung metastases were present in 33 of 144 mice (23%) in 3 experiments. They were located at both the surface and depth of the organ and ranged in size from microscopic formations to growths 3 mm. in diameter, at times confluent with other growths of comparable size. In Experiments A and B1 the metastatic lesions were usually multiple and invasive, whereas in Experiment B2, begun a year later, the lesions tended to be solitary and less invasive. Nevertheless, the incidence of metastases remained about the same throughout all passages and the microscopic appearance of the primary tumors was maintained in the metastatic lesions, even though necrosis was more prevalent among the larger primary growths (Figs. 4, 5, 6, 7).

Gross inflammatory lesions of the viscera developed in 21 of the 34 mice (61%) comprising the first 5 passages of Experiment A, in which lyophilized tissue was added to the inoculum, and in 6 of the 33 mice (18%) of Experiment B1. In no instance was the inoculum treated with antibiotics. There were no similar lesions found from the 6th to the last passage of Experiment A, nor in any of the passages of Experiment B2, in which antibiotic was added to the inoculum. Therefore, the incidence of these inflammatory lesions diminished considerably when inoculations of lyophilized tissue were omitted from the passages and disappeared altogether when antibiotics were added to the inoculum of tumor cells. The lesions were most noticeable in the liver, which was grossly enlarged and characteristically ragged and mottled. The spleen, lymph nodes, and other tissues were also enlarged and discolored, whereas the lung lesions were predominantly hemorrhagic. Microscopically, the liver contained large nodules of polymorphonuclear leucocytes and other inflammatory cells, which were usually located perivascularly and associated with diffuse or focal necrosis. Comparable lesions were observed in the spleen, lymph nodes, and other tissues. In fact, those mice devoid of gross inflammatory lesions, which had received an inoculum without antibiotic, frequently had microscopic lesions which were readily detected. The gross lesions can, therefore, be regarded as an exponent of the total incidence of infection (Figs. 8, 9, 10). In the bacteriological investigation of the inflammatory lesions and blood of infected mice, coliform organisms were recovered consistently. To estimate the virulence of these bacteria, normal mice were injected with representative cultures. The minimal lesions produced indicated a very low pathogenicity indeed.

TABLE 2. OCCURRENCE OF METASTASES AND INFLAMMATORY LESIONS IN MICE TREATED WITH CORTISONE AND GRAFTED WITH HAS TUMOR

| | *Cortisone with or without lyophilized material (A series)* | | | | | | *Cortisone exclusively* | | | | | | *Untreated controls* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Metastases in lungs* | | *Inflammatory lesions in viscera* | *Average survival days* | | | *Metastases in lungs* | | *Inflammatory lesions in viscera* | *Average survival days* | | | | *Inflammatory lesions* | *Average survival days* |
| *Passage* | *No. mice* | *Incidence* | *Severity* | | | *Passage* | *No. mice* | *Incidence* | *Severity* | | | *Passage* | *No. mice* | *Metastases* | | |
| Exp. A | | | | | | Exp. B1 | | | | | | | | | | |
| 1 | 8 | 2 | ++ | 5 | 35 | 1 | 7 | 2 | +++++ | 3 | 33 | 1 | 8 | 0 | 0 | |
| 2 | 9 | 5 | +++++ | 7 | 34 | 2 | 8 | 1 | + | 1 | 32 | 2 | 6 | 0 | 0 | 46 |
| 3 | 7 | 1 | + | 4 | 30 | 3 | 8 | 4 | ++++ | 1 | 39 | 3 | 10 | 0 | 0 | |
| 4 | 8 | 2 | ++ | 3 | 31 | 4 | 10 | 0 | | 1 | | 4 | 9 | 0 | 0 | |
| 5 | 2 | 1 | + | 2 | 41 | | | | | | | 5 | 10 | 0 | 0 | |
| Total* | 34 | 11 (30%) | | 21 (61%) | | | 33 | 7 (21%) | | 6 (18%) | | | 43 | | | |
| 6 | 6 | 1 | ++ | 0 | 30 | | | | | | | 6 | 8 | 0 | 0 | |
| 7 | 2 | 0 | | 0 | 37 | | | | | | | 7 | 10 | 0 | 0 | |
| 8 | 3 | 0 | | 0 | 37 | | | | | | | 8 | 10 | 0 | 0 | 44 |
| 9 | 6 | 1 | ++ | 0 | | | | | | | | 9 | 10 | 0 | 0 | |
| 10 | 1 | 0 | | 0 | | | | | | | | 10 | 10 | 0 | 0 | |
| 11 | 5 | 2 | +++++ | 0 | 56 | | | | | | | 11 | | | | 33 |
| 12 | 8 | 2 | ++ | 0 | 46 | | | | | | | 12 | | | | 34 |
| | | | | | | Exp. B2 | | | | | | | | | | |
| 13 | 5 | 0 | | 0 | 48 | 1 | 3 | 0 | | 0 | 43 | 13 | 4 | | | 50 |
| 14 | 7 | 1 | ++ | 0 | 47 | 2 | 5 | 0 | | 0 | 46 | 14 | 5 | 0 | 0 | 35 |
| 15 | 3 | 1 | ++ | 0 | 42 | 3 | 4 | 1 | + | 0 | 43 | 15 | 2 | 0 | 0 | 48 |
| 16 | 4 | 1 | ++ | 0 | 47 | 4 | 4 | 1 | + | 0 | 47 | 16 | 4 | 0 | 0 | 39 |
| 17 | 6 | 2 | ++ | 0 | 36 | 5 | 5 | 2 | + | 0 | 43 | | | | | |
| Total | 55 | 11 (20%) | | 0 (0%) | | | 21 | 4 (19%) | | 0 (0%) | | | 63 | | | |
| Grand total | 90 | 22 (24%) | | | | | 54 | 11 (20%) | | | | | 106 | 0 (0%) | 0 (0%) | |

* Injection of lyophilized tissue discontinued.

From the data presented thus far, it is evident that cortisone inhibits the growth of the primary HAS tumor while it simultaneously promotes the development of metastases in the lung and inflammatory lesions in the viscera. These phenomena have never been observed in 8 years of routine transplantation without the hormone. With cortisone they are manifest from the first passage, but are not increased by additional passages—up to 17—in cortisone-treated mice or by complementary injections of lyophilized tumor tissue. The inflammatory lesions which were found to co-exist with tumor metastases in the lungs and other viscera, initiated by latent bacteria of low virulence, nevertheless gave the gross appearance of a generalized leucotic infiltration subsequent to the growth of the primary tumor.

*Tumors passed from cortisone-treated to nontreated hosts.* To ascertain whether the acquired property of metastases and the association with inflammatory lesions would persist in the absence of cortisone, the tumors from cortisone-treated animals were transplanted to untreated C57 black mice. Cell suspensions from 7 different primary tumors in 4 passages of the A series were inoculated into untreated mice without the admixture of antibiotics. With 2 sub-lines thus started, 3 passages in 3 months and 16 passages in 12 months were continued through other untreated mice in exactly the same manner as with the A and B lines. Metastases never developed. On the other hand, as shown in Table 3, gross generalized inflammatory lesions did occur which, in one passage, proved to be lethal for most mice. However, the generalized inflammatory lesions faded in the course of the passages even though bacteria continued to thrive in the primary tumors, as demonstrated by culture.

In another experiment, tumor tissue from lung metastases was prepared in the same manner as primary tumor suspensions, and the metastatic cells were inoculated into 10 cortisone-treated mice and 10 normal controls. Tumors developed in all mice, and lung metastases developed in 2 animals treated with cortisone.

From these experiments it is evident that the development of lung metastases and of inflammatory lesions does not persist in passages through untreated mice.

*Passage of normal liver and spleen extracts through cortisone-treated mice.* Although the above results clearly implicate the bacterial flora of the tumor in the occurrence of inflammatory lesions, additional experiments were undertaken. To parallel the first tumor passages in Experiments A and B1, 10 mice were prepared by the usual intraperitoneal injection of lyophilized tumor tissue and were then grafted with the sarcoma. The tumor grew in the prepared hosts as in normal mice, but 4 of the animals

TABLE 3. EFFECT OF PASSAGES THROUGH UNTREATED MICE OF TUMOR FROM CORTISONE-TREATED LINES

| *Source tumor from cortisone-treated mice (A series)* | *Sub-lines* | *1st passage* | | *2d passage* | | *3d passage* | | *4th passage* | | *5th-16th passage* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *No. mice* | *Inflammatory lesions* | *No. mice* | *Inflammatory lesions* | *No. mice* | *Inflammatory lesions* | *No. mice* | *Inflammatory lesions* | *No. mice* | *Inflammatory lesions* |
| From 1st passage | 1 | 10 | 2 | | | | | | | | |
| From 2d passage | 1 | 10 | 3 | | | | | | | | |
| | 2 | 5 | 2 | 5 | 2 | 10 | 0 | | | | |
| | 3 | 10 | 10 | 7 | 7 | 2 | 2* | 4 | 0 | 60 | 0 |
| From 4th passage | 1 | 5 | 2 | | | | | | | | |
| | 2 | 5 | 0 | | | | | | | | |
| From 6th passage | 1 | 10 | 0 | | | | | | | | |

* Another 8 mice failed to grow tumor due to lethal generalized infection.

receiving lyophilized extract developed gross inflammatory lesions of the viscera. In a second experiment the possibility of reviving latent bacteria in the tissues of normal C57 mice was investigated by passage through cortisone-treated mice. A total of 4 passages was carried out approximately at monthly intervals, using a 1 : 20 cell suspension of normal liver or spleen injected subcutaneously into 4 mice. In not one instance was a gross or microscopic inflammatory lesion observed in the viscera nor were bacteria recovered from the inocula.

*Cell-free HAS preparation and tumor production.* The transmissibility of the sarcoma by filtrates or desiccates has been tested repeatedly in newborn and adult C57 black mice since the earliest passages. The results have been consistently negative. Experiments were repeated in the current study using filtrates from a wide range of tissues. On 16 different occasions the primary tumors, the metastases, and the livers and spleens of mice treated with cortisone were extracted with 10 volumes of saline and centrifuged, yielding extracts which could be filtered through a Berkefeld N. candle. Aliquots of the filtrate ranging from 0.1-1.0 ml. have been injected into approximately 150 C57 black mice ranging in age from a few hours to 6 weeks. In many instances the mice had previous treatment with cortisone, some with and others without lyophilized tumor. In some of the newborn mice cortisone was injected subcutaneously with the filtrate. The injected animals were kept for 2-5 months. In not one instance was a tumor induced nor did a lesion of any sort develop.

## DISCUSSION

Pertinent to our study and the interpretation of results is the work of the Chilean observers, Agosin, *et al.*,[1] Christen, *et al.*[10] and Tallman and Gasic,[45] who used an adenocarcinoma grown in cortisone-treated C57 mice, the homologous strain, to show a marked suppression of the primary tumor growth with the concomitant development of metastases. The effect persisted only in treated animals and grafting of the metastatic tissue induced ordinary tumors. Similar observations were made by Baserga and Shubik[5, 6] in an adenocarcinoma from DBA mice. Others, such as Higgins,[20] Antopol, *et al.*,[4] Martinez, *et al.*,[31] Gottschalk,[19] and Stock, *et al.*,[43] described and discussed the suppressing effect of the hormone on the primary growth of a variety of tumors, while Pomeroy[38] studied the effects of cortisone treatment on tumor cells injected intravenously. The action of the hormone has been variously and tentatively interpreted as being exerted directly on the tumor cell,[20, 21] or indirectly on the tumor and variably directed at its metastases;[5, 20, 38] it is said by others[2] to exert an effect on the mesenchyme, particularly its ground substance. Discrepancies in observations have been

variously attributed to the intrinsic characteristics of the tumor, to dosage or time of cortisone injection, and conceivably to other factors.

Concerning the effect of cortisone on saprophytic bacteria, Selye[40] provoked a disease in rats which is very similar to that which we have described in mice. He has speculated on changes in the ground substance of the mesenchyme which would influence the critical concentration of bacteria and the inducement of tissue invasion.[38]

The results presented in this paper deal with cortisone-treated mice bearing transplants of a chemically induced tumor, and confirm these recent studies using different techniques and hosts.* The phenomena observed include the inhibition of primary tumor growth with the simultaneous induction of metastases,[2, 5, 6] and the provocation of generalized infection by latent saprophytic bacteria.[37, 40, 44] However, the significance of the present experiments has been to compare these two phenomena and observe their simultaneous occurrence in one host. Thus, the bacterium and the cancer cell can be observed while subject to the same influences, and the behavior of one cell can be evaluated in the light of what is known about the other.

Both phenomena under consideration depend upon the persistent presence of cortisone, for metastases as well as generalized bacterial lesions do not occur after a few passages of the tumors through normal mice. Hence, no permanent variation toward increased malignancy or virulence had taken place in either cancer or bacterial cells under the effect of the hormone; indeed, when returned to normal mice, both cancer cells and bacteria soon resumed their existence as in ordinary passages. Nor was the effect of cortisone cumulative for either type of cell, because repeated passages of the tumor through the hormone-treated animals did not increase the incidence of metastases or of bacterial lesions above that observed in the first passages.

Concerning the effect of cortisone on the growth of the primary tumor, it is pertinent to consider the work of Lurie[29, 30] dealing with the influence of the hormone on tubercular infections in rabbits. One of the main points he disclosed was inhibition of the primary foci of infection, resulting in smaller tubercles and restricted invasion of adjacent structures. This can be attributed to decreased permeability of capillaries[29, 30] and ground substance.[8, 9, 29, 48] Is it possible that the pronounced, albeit temporary, inhibition of the primary tumor by cortisone could be a direct counterpart of the phenomenon Lurie described?

Concerning the formation of metastases, it is feasible to consider solitary cells detached from the mass of the primary growth, their dissemination in

* The present investigation was started in 1952 before the results of the effect of cortisone on tumors[2, 5, 6] were known.

the blood, and their nidation in remote tissues as events analogous to septicemia and the formation of secondary abscesses. In the present study both cancer cells and bacteria, living in close approximation in the primary tumor, were disseminated widely throughout the viscera. A second analogy with bacterial infection may be justified if one considers the capacity of cortisone[27, 30] to increase the phagocytic power of macrophages, leading to rapid fixation of circulating mycobacteria and carbon particles, while at the same time the digestion of bacteria by the phagocytic macrophages is blocked, leading to unrestricted bacillary multiplication. It is possible that enhanced phagocytic function results in fixation and multiplication of the blood-borne cancer cells as well as bacteria, thus leading to metastasis as well as to abscess formation. Two studies are particularly pertinent to this concept: one is that of Pomeroy,[28] which shows that tumor cells injected intravenously induce growths with greater frequency in cortisone-treated mice; and the other is that of Kass, *et al.*,[24] which indicates that the hormone impairs the digestive capacity of the macrophage for red blood cells which are practically as large as tumor cells and larger than bacteria.

The parallel behavior of bacterial and cancer cells is further emphasized by examining the effects of estrone, a hormone in many ways antagonistic to cortisone. On the one hand, estrone strongly protects against streptococcal,[17] pneumococcal,[47] and tubercular[29, 30] infection, and on the other hand it markedly suppresses the growth of transplantable tumors in animals[21, 28] and, to a certain extent, metastatic malignancy in humans.[1, 26, 46] The hormone has the property of stimulating the formation of large amounts of ground substance in monkeys[14] and in some strains of mice.[39] The effects of estrone on infection are due, at least in large measure, to phenomena occurring in the ground substance,[18, 29, 42] and studies presently carried out in our laboratory on Swiss mice—hosts responding equally well to the hormone[15]—give strong evidence for the same mechanism with cancer.[18] Cortisone also induces effects on the ground substance, sometimes of a contradictory nature. These effects are manifested in both normal and pathological tissues by depletion of the whole intercellular matrix,[8, 9, 37] and consequent increase or decrease of the barrier value leading to changes in the spreading reaction.*[37, 45]

Whether, in hosts in a state of endocrine imbalance, the analogies between bacterial infection and the growth of malignancy are only of a superficial sort, or whether they reveal a fundamental identity in the behavior of bac-

* As examples of further analogies between the behavior of bacterial cells, one can well quote the work of Murphy;[34] the effect of lymphocytic reaction on cancer and tuberculosis can be considered either in itself or as an index of a more general reaction of the organism.

terial and cancer cells, is not apparent. One can state, however, that further studies concerning the effect of hormones on the mesenchyme of the host and on the ground substance of the tumor itself are likely to be fruitful; it is an accepted fact that events concerned with the cohesion of matrices of the tumor[11,12]—and surrounding host tissues as well[9,10,6]—are of great importance in the formation of metastatic lesions. The tumor used in the present study—the HAS sarcoma—is extremely rich in ground substance, and changes induced in it by hyaluronidase are readily ascertained by histochemical methods. One could expect that the tumor would also reflect hormonal effects exerted on its ground substance, as well as in the mesenchyme of the host. Studies in this direction are contemplated.

## SUMMARY

Under the effect of cortisone, a transplantable mouse sarcoma, which has never been known to induce metastases in normal hosts, gave rise to metastatic lesions during the simultaneous, although temporary, inhibition of the primary tumor.

Concurrent with the above phenomena, pronounced inflammatory lesions occurred in the viscera due to generalized infection with saprophytic bacteria present in the tumor. Both effects were manifest from the first passage and did not increase during further passages.

Both effects of the hormone disappeared upon passage of either the primary tumors or metastatic lesions through normal mice. The effect of bacterial infection could be suppressed by antibiotic treatment even in cortisone-treated mice.

The methylcholanthrene-induced sarcoma used in these studies (HAS) is extremely rich in viscid ground substance which is attacked by testicular and bacterial hyaluronidase. This tumor has been described, and its possible usefulness as a tool to study metastases formation and other phases of malignancy has been indicated.

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# CARDIAC AND HEPATIC LESIONS IN MICE FED YEAST-PROTEIN DIETS
## I. DIETS CONTAINING BRITISH BAKERS YEAST‡

## INTRODUCTION

Massive necrosis of the liver occurs in rats restricted to diets in which yeast is the sole source of protein.[1-4] The present report considers the cardiac and hepatic lesions that developed in mice fed diets in which British yeast was the source of protein. In addition, studies have been made of the possible protective actions of dietary supplements (cystine or a-tocopherol) and of the substitution of casein for a portion of the yeast content of the diet. British bakers yeast was used because it has frequently served as the protein component of diets that produced a high incidence of hepatic necrosis in rats.[11, 14-17, 19]

## MATERIALS AND METHODS

*Animals:* Mice of the AB stock (Strong A x Bagg albino) were restricted to the yeast diets when 10 weeks of age. From the time of weaning (30 days) these mice had been fed the stock colony diet of Purina Fox Chow. Males and females were used in approximately equal numbers.

*Diets:* The composition of the diets was as follows:

I. British yeast diet (BYD), 114 mice:

| | |
|---|---|
| British bakers yeast§ | 180 gm. |
| Corn starch | 666.5 |
| Salt mixture (No. 2, U.S.P. XIII) | 40 |
| Peanut oil | 100 |
| Cod liver oil | 4.5 |
| Vitamin powder (see below) | 10 |

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§ Dried Baking Yeast (United Yeast Co., Ltd., London, England).

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| | |
|---|---|
| Vitamin powder: | |
| Thiamine | 0.5 gm. |
| Riboflavin | 0.25 |
| Ca. pantothenate | 1.0 |
| Pyridoxine | 0.2 |
| Nicotinic acid | 0.2 |
| Powdered sugar | 997.05 |

II. BYD plus vitamin E, 45 mice:

These mice fed the British yeast diet (BYD) also received vitamin E as an intramuscular injection of 2.5 mg. of a-tocopherol in saline solution twice weekly.*

III. BYD plus cystine, 25 mice:

Cystine (l-cystine) was added to the standard BYD diet at the level of 5 gm. per 1,000 gm. of diet.

IV. BYD plus casein, 44 mice:

Casein ("vitamin free") was substituted for one-half of the British yeast content of the standard diet. In this diet the yeast content was 9% and the casein content 9%.

*Histological methods:* Tissues were fixed in a modified[42] Lavdowsky's solution (alcohol, formalin, and acetic acid) except for the demonstration of lipids when 10% formaldehyde was used. Following use of the latter, frozen sections were cut and were stained with sudan IV or sudan black B. Methods for demonstrating fibrosis (Masson or Mallory), minerals (von Kossa), and PA-S- (periodic acid-Schiff's reagent) positive material were used as described by Lillie.[25] Basophilia and cytoplasmic ribonucleic acid of hepatic parenchyma were demonstrated by use of ribonuclease-hydrolysis and staining with basic dyes as described in an earlier study.[43]

## RESULTS

*Controls.* All mice of this group were fed Purina Fox Chow. One hundred mice, 4 to 8 months of age, of the AB stock showed no cardiac, hepatic, or skeletal muscle lesions. In 46 mice of the same stock, 9 to 16 months of age, evidence of cardiac damage was limited to a small fibrotic area in one heart. Livers and muscle were normal. Diffuse pneumonia was a frequent "natural" cause of death in these mice.

*Mice fed British yeast diets.* The data concerning incidence of lesions are presented in Tables 1 and 2. The results discussed here are chiefly those related to the histopathological aspects of the lesions. The mice fed the diet in which one-half of the yeast was replaced by casein (BYD plus casein diet) were free of significant cardiac or hepatic lesions, but pneumonia was present in most of those that died. Cytoplasmic granulation, acidophilic and

* Generously supplied by Dr. Philip L. Harris of the Distillation Products Industries, Rochester, New York.

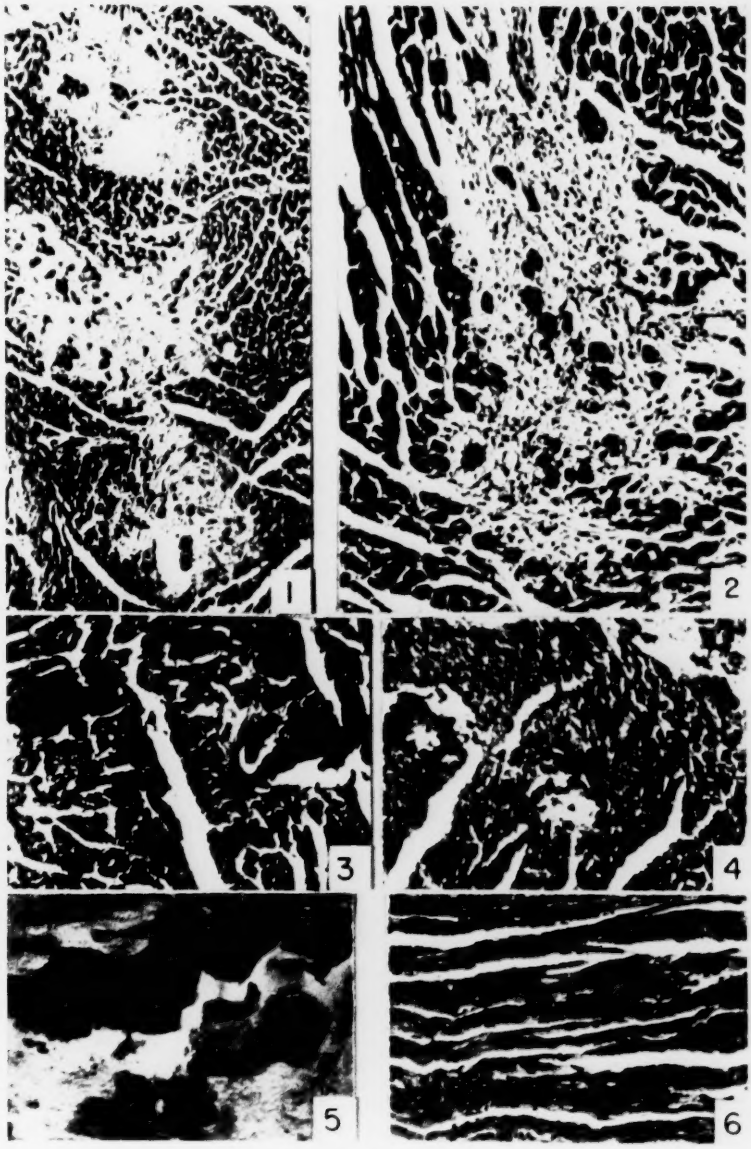



PLATE I

All figures of 12 rat hearts or livers of rats fed the nonsupplemented Burch yeast diet (BYD).

FIG. 1. Three areas of myocardial fibrosis. Masson. ×100.

FIG. 2. An area of fibrosis as shown in Figure 1. The dark round structures are cardiac muscle fibers. These areas contain no deposits of mineral or cetoid. Masson. ×150.

FIG. 3. Two deposits of mineral cetoid in myocardium. von Kossa. ×150.

FIG. 4. Same material as in Figure 3 except that the section was "demineralized" in nitric acid prior to application of the von Kossa technique. The intensity of the staining reaction is greatly reduced. ×150.

FIG. 5. Mineral cetoid material as shown in Figures 3 and 4. A routine paraffin-embedded section stained with solan black B. This staining (plus acid fast and PAS positivity) indicates that the material contains cetoid. ×400.

FIG. 6. The myocardium is normal except for deposits of basophilic material. H and E. ×150.

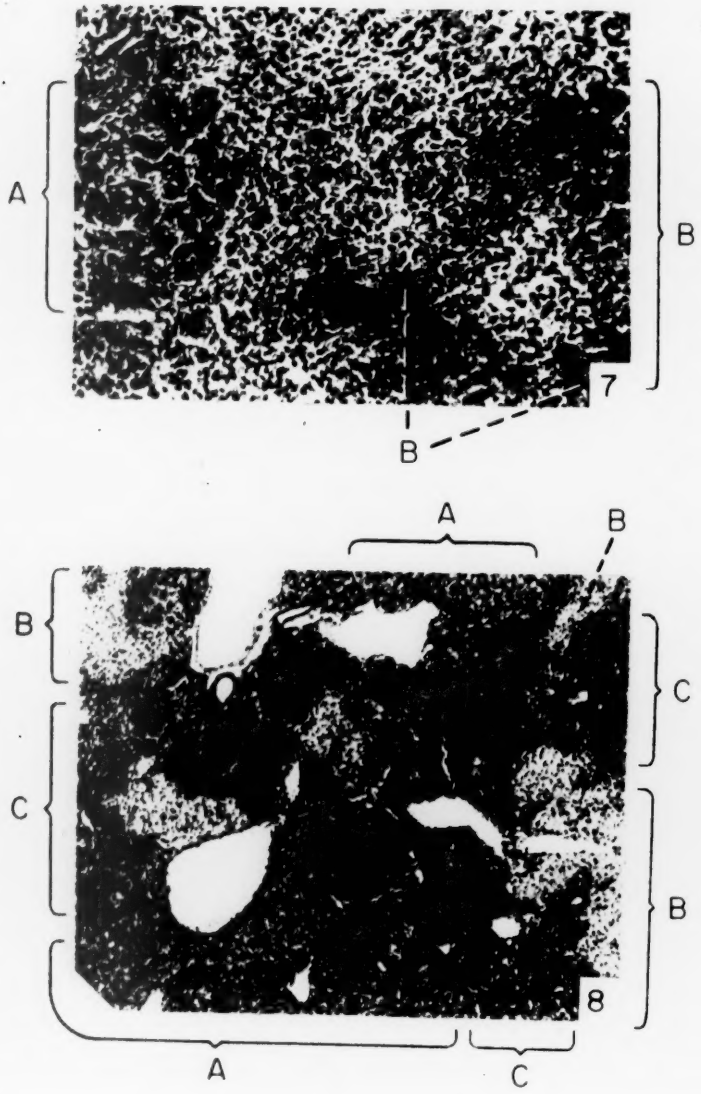
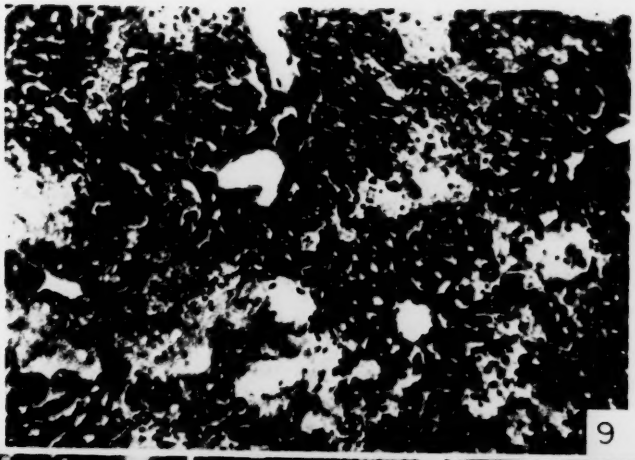



PLATE 2.

FIG. 7. Areas of relatively normal hepatic parenchyma (A) and of acute necrosis (B). The remainder of the section contains many small cells (macrophages, lymphocytes, and fibroblasts) which seem to be located in sites of earlier necrosis. H and E; ×150.

FIG. 8. Liver stained with gallocyanin-chromalum (at pH 2.5). Maximum cytoplasmic basophilia exists in areas of relatively intact parenchyma (A). Cytoplasmic basophilia is negligible in sites of active necrosis (B). Previous episodes of necrosis are indicated by the areas (C) containing many small cells (as in Fig. 7). ×50.



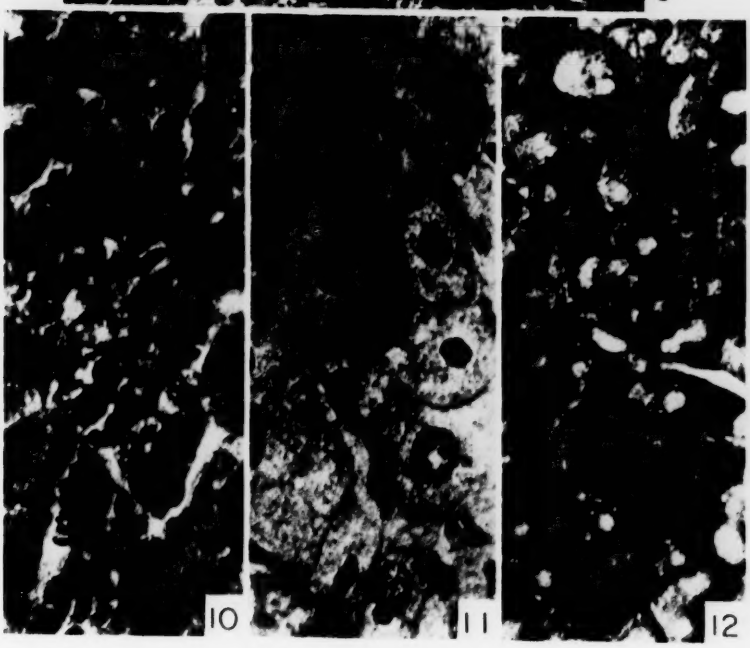




PLATE 3

FIG. 9. Areas of intact hepatic parenchyma and of active necrosis (H. and E.).

FIG. 10. Normal liver stained with toluidine blue. The cytoplasm of the parenchymal cells shows the characteristic coarse, granular basophilia. ×700.

FIG. 11. Area of "intact" parenchyma in liver showing necrosis. The cytoplasm is extremely hypobasophilic. Toluidine blue. ×700.

FIG. 12. Same area as in Figure 11, but stained by the PAS technique to show glycogen. The amount of PAS positivity approximates that of a normal liver. ×700.

basophilic, of hepatic parenchyma was slightly reduced. Necrosis was not observed in hearts or livers.

In the other mice the histological characteristics of the lesions could not be correlated with the diet (BYD, BYD plus vitamin E, BYD plus cystine). The incidence of cardiac lesions was decreased in the vitamin E group and that of hepatic and myocardial necrosis increased in the cystine-supplemented group (see Tables 1 and 2).

TABLE 1. FINDINGS IN MICE FED THE BRITISH YEAST DIETS (BYD)

| | *The four BYD diets used:* | | | |
|---|---|---|---|---|
| | *BYD*<br>*114 mice* | *BYD plus casein*<br>*41 mice* | *BYD plus vitamin E*<br>*45 mice* | *BYD plus cystine*<br>*25 mice* |
| *No hepatic necrosis or myocardial lesions:* | 30% | 100% | 64% | 20% |
| *Days* on diet: | | | | |
| Average | 55 | 228 | 49 | 22 |
| Range | 7–201 | 30–283 | 14–167 | 14–63 |
| *With myocardial lesions:* | 69% | 0% | 31% | 76% |
| *Days* on diet: | | | | |
| Average | 97 | ..... | 97 | 62 |
| Range | 30–283 | ..... | 30–158 | 14–112 |
| *With hepatic necrosis:* | 8% | 0% | 11% | 52% |
| *Days* on diet: | | | | |
| Average | 153 | ..... | 124 | 59 |
| Range | 60–272 | ..... | 109–134 | 7–112 |

NOTE: In a given dietary group the total per cent in excess of 100 is due to occurrence of both myocardial and hepatic lesions in some of the mice.

Mice fed the yeast diets (except the casein-supplemented group) weighed approximately 20% less than controls of the same age. Their coats were scanty and rough. These mice were generally inactive except upon manual or auditory stimulus when they were very hyperactive.

*Hearts.* The lesions were of two basic types, the incidence of each was approximately the same, and there was no evidence that one was the antecedent of the other. Obvious myocardial necrosis and fibrosis (see Figs. 1 and 2) represented one type. The other consisted of intramyocardial deposition of material that was basophilic and was also PA-S-, von Kossa-, and ceroid-positive (see Figs. 3-6). Fibrosis had occurred around these deposits. Although nitric acid was used to demineralize such areas it was

TABLE 2. INCIDENCE OF LESIONS (AS PER CENT OF TOTAL NO. OF MICE, KILLED OR DIED, DURING THE QUARTER); AND PER CENT OF TOTAL MORTALITY DURING QUARTER

| | *British yeast diets (BYD)* | | | |
|---|---|---|---|---|
| | *BYD 114 mice* | | *BYD plus vitamin E* | *BYD plus cystine* |
| | *33 mice killed* | *81 mice died* | *45 mice (all died)* | *25 mice (all died)* |
| First quarter (1-13 weeks) | *24 mice* | *58 mice* | *30 mice* | *22 mice* |
| No hepatic necrosis or myocardial lesions | 29% (49*) | 40% (44*) | 83% (37*) | 18% (28*) |
| Myocardial lesions | 67% (69*) | 59% (64*) | 17% (39*) | 73% (54*) |
| Hepatic necrosis | 4% (64*) | 3% (64*) | 0% | 55% (45*) |
| Mortality | ..... | 72% (56*) | 67% (38*) | 88% (46*) |
| Second quarter (14-26 weeks) | *4 mice* | *19 mice* | *15 mice* | *3 mice* |
| No hepatic necrosis or myocardial lesions | 75% (112*) | 0% | 27% (123*) | 0% |
| Myocardial lesions | 25% (100*) | 100% (115*) | 60% (129*) | 100% (103*) |
| Hepatic necrosis | 0% | 1% (110*) | 33% (124*) | 33% (112*) |
| Mortality | ..... | 23% (115*) | 33% (126*) | 12% (103*) |
| Third quarter (27-39 weeks) | *4 mice* | *4 mice* | | |
| No hepatic necrosis or myocardial lesions | 0% | 25% (201*) | No | No |
| Myocardial lesions | 100% (243*) | 75% (233*) | Survivors | Survivors |
| Hepatic necrosis | 25% (272*) | 75% (233*) | | |
| Mortality | ..... | 5% (225*) | | |
| Fourth quarter (40-52 weeks) | *1 mouse* | | | |
| No hepatic necrosis or myocardial lesions | 0% | No | No | No |
| Myocardial lesions | 100% (283*) | Survivors | Survivors | Survivors |
| Hepatic necrosis | 0% | | | |
| Mortality | ..... | | | |

* Average number of days on diet.

not possible to demonstrate myocardial necrosis within them. The surrounding fibrosis could indicate either replacement of necrotic myocardium or a reaction of the foreign body type to the mineral-ceriod deposits. Following exposure of the paraffin sections to 5% aqueous nitric acid, the reaction to the von Kossa technique was very faint (see Figs. 4 and 6), and the intensity of the PA-S reaction was diminished. The smallest of these deposits were basophilic (see Fig. 6) and PA-S-positive, but did not react to the von Kossa procedure. However, some mineral may have been removed by the acidic solution in which the tissues were fixed. The type of myocardial lesion did not seem to be related to the duration of restriction to the diet or to the presence or absence of vitamin E or cystine supplement.

The ventricles were the usual site of both types of lesions, otherwise there was a limited incidence of pericarditis and of diffuse myocarditis. Endocarditis and valvulitis were not observed.

*Livers.* The areas of necrosis usually involved several lobules (see Figs. 7-9). The parenchyma surrounding or adjacent to foci of necrosis appeared relatively intact in routine preparations stained with hematoxylin and eosin. Application of the ribonuclease hydrolysis-basophilic staining technique and of the gallocyanin-chromalum staining procedure[23] demonstrated a depletion of cytoplasmic ribonucleic acid in these parenchymal cells (see Figs. 8, 10, 11). Glycogen (PA-S technique) was not decreased significantly (see Fig. 12) and fat (sudanophilia of frozen sections) was not obviously increased in their cytoplasm. Neither cirrhosis nor ceroid was observed in the livers. In the livers of a very small number of mice (less than 1%) there were areas containing macrophages, lymphocytes, and a few neutrophils. These areas (see Figs. 7 and 8) were outside of the currently active sites of necrosis. Some of the cells may have been fibroblasts, but no fibres or ground substance could be demonstrated (see Fig. 7). Areas of similar appearance and composition in rats have been described as representing sites of previous episodes of dietary induced hepatic necrosis.[23] Mitoses were frequent in the areas of intact parenchyma, but the dividing cells were not adjacent to foci of necrosis. Often they seemed to be located in the centres of islands of fairly healthy parenchyma.

*Skeletal muscle.* It is obviously difficult to survey the voluntary musculature even in an animal as small as a mouse. Thigh muscles were studied routinely and in some instances, perivertebral muscles. In approximately one-half of the mice with cardiac lesions, the voluntary muscles contained mineral-ceroid identical with that described above as in the myocardium. Deposition of such material was not prevented by the vitamin E or cystine supplements.

*Lungs.* Diffuse pneumonia was observed in all animals that had cardiac or hepatic lesions. All animals that died showed pneumonia.

*Reproductive tract.* The seminiferous epithelium was generally atrophic except in the mice fed the diet in which casein had been substituted for one-half of the yeast. Spermatogenesis had not advanced beyond early spermatid forms. The type of tubular degeneration that occurs in vitamin E deficient rats[29] was not observed in the testes. Ovaries and uteri did not show deposition of ceroid that indicates vitamin E deficiency in the rat.[29, 30]

*Other systems.* The kidneys, adrenals, blood vessels, and peripheral nerves appeared normal except for renal abscesses in two mice. Adequate studies of the alimentary and central nervous systems were not made. Bones appeared normal in routine, decalcified sections stained with hematoxylin and eosin. Smooth muscle seemed normal. Oral and dental structures were not studied. The system of lymphatic organs showed no significant changes except a depletion in the number of mature lymphocytes which occurs in dietary restriction.

## DISCUSSION

The results of this study demonstrate that in mice there exists a greater myocardial than hepatic susceptibility to the injurious effects of the yeast-containing diets. The histological pattern of necrosis in the mouse liver was similar to that observed in rats fed yeast-protein diet.[1-4, 14, 23] The frequency of hepatic necrosis in mice was approximately 10% of that observed in rats fed the same diet except in the cystine-supplemented group that attained more than one-half of the incidence in rats.[14, 17] The predominance of myocardial lesions in mice is of further importance because of the high incidence of such lesions in the same species[18] when fed a protein deficient diet.

Deficiencies of sulphur-containing amino acids (cystine and methionine)[8, 21, 22, 41] and of vitamin E favor the development of massive hepatic necrosis.[6, 24, 28, 40] Supplementation to eliminate these deficiencies decreases the incidence or prolongs the time of onset of hepatic injury.[16, 17, 24, 35-38] The relation of potential or real deficiencies of specific amino acids and of vitamin E in the induction of these lesions is further complicated by the marginal if not definitely inadequate amount of total protein (7%) in these diets.[18, 34-36]

Supplements of vitamin E decreased the total incidence of cardiac lesions without significantly altering that of hepatic necrosis (see Tables 1 and 2). Lesions of voluntary and of cardiac muscle occur in rats fed diets that are deficient in vitamin E.[9, 10, 13, 27, 30, 32] The synergistic or mutual effects of co-

existent protein and vitamin E deficiencies must be considered as causes of muscle lesions (cardiac or skeletal) and of hepatic necrosis.[7,31] Prefeeding of rats with vitamin E and sulphur containing amino acids protects against subsequent production of hepatic lesions by feeding protein deficient diets.[28] Also, a low protein diet accelerates the injurious action of vitamin E deficiency upon the liver and voluntary muscles.[12]

The nutritional basis of the lesions observed here in mice fed the British yeast diet may be distinctly different from that responsible for the hepatic necrosis that occurs in rats fed the same diet.[14,17,28] The present data showing in mice a predominance of cardiac lesions and a relative infrequency of hepatic necrosis indicate some difference. Supplements of vitamin E or of cystine were completely effective in preventing hepatic necrosis in rats fed the British yeast diet for as long as 200 days.[15] In the mice vitamin E was only moderately "protective" for a limited time (see Table 2) and supplementation with cystine increased the necrogenicity of the diet in relation to both the heart and liver. Small supplements of cystine are known to exert "toxic" effects when concurrent deficiencies of lipotropic factors and amino acid sulphur exist.[5] However, the changes in the livers of the mice, particularly the lack of both significant intracytoplasmic liposis within the parenchyma and of cirrhosis, do not indicate a deficiency of lipotropic factors.

The feeding of casein-supplemented diets to the mice demonstrated that the presence of yeast in the ration was not in itself necrogenic to the liver and heart.

Hepatic necrosis has been produced in rats by feeding diets in which a considerable number of different yeasts served as the source of protein.[1,4,24-26] The relevance of data furnished by such studies to the nutritional needs of mice is not known. The study of British yeast reported here and a preliminary survey[29] of two strains of American yeast* show that in mice the myocardium is highly susceptible to injury produced by deficiencies existing in diets in which yeast is the source of protein.

## SUMMARY

When 70 days of age, 228 albino mice were restricted to a basic diet in which the sole source of protein was British bakers yeast. Of these, 45 mice received supplements of vitamin E (intramuscularly); 25 were fed the basic diet plus cystine (0.5 gm./100 gm. diet); and 44 were fed the

* Strains K2 and G of *Saccharomyces cerevisiae* yeasts furnished through the courtesy of the Central Research Department of Anheuser-Busch, Inc., St. Louis, Missouri.

basic diet except for substitution of "vitamin free" casein for one-half of the yeast content.

The remaining mice (114) were fed the basic diet without supplement of vitamin E, cystine, or casein. Within 97 days (average) 69% of these animals showed myocardial lesions. The incidence of hepatic necrosis was 8% at an average of 153 days.

Supplementation with vitamin E reduced the incidence of myocardial lesions without significantly altering the frequency of hepatic necrosis.

Addition of cystine increased both cardiac injury (76% incidence within 62 days) and hepatic necrosis (52% incidence within 50 days).

Mice of the casein-supplemented group were killed within 30 to 283 days. No significant lesions were observed.

The histological type of hepatic necrosis was similar to that observed in rats fed yeast diets. Myocardial lesions included necrosis, fibrosis, and deposition of ceroid and mineral (von Kossa) within damaged fibres. At least one-half of the mice with myocardial lesions also showed deposition of ceroid-mineral within skeletal muscle fibres. Livers did not show liposis, cirrhosis, or deposition of ceroid pigment.

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*The Board of Editors*

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# NEURAMINIC ACID: THE FUNCTIONAL GROUP OF SOME BIOLOGICALLY ACTIVE MUCOPROTEINS

Present knowledge would attribute to proteins a wider range of biological activity than is possessed by any other group of natural substances. Thus enzymes, respiratory pigments, antibodies, many antigens, and several hormones are protein in nature. Sometimes the biologically active substance is a simple protein, consisting of amino acid residues only (papain, carboxypeptidase, lysozyme, insulin, glucagon, $\beta$-corticotropin, etc.). In other cases the protein is conjugated with nonprotein material as in the photosynthetic pigments, respiratory enzymes, the oxygen carriers haemoglobin and chlorocruorin, the gonadotropic hormones, Castle's intrinsic factor, blood group substances, virus haemagglutinin inhibitors, and others. Whereas it is well established that the haem iron in haematoproteins provides the active centre, and that flavin adenine mononucleotide and dinucleotide, respectively, are the functional groups of catalytically active flavoproteins, the literature provides little, if any, information about the structural element responsible for the biological activity of mucoproteins, i.e., carbohydrate-protein complexes with high protein content.

Over the past nine years the staff of this Institute has been engaged in an investigation of the interaction between the influenza virus particle and some mucoproteins at both the biological and the biochemical levels. These investigations, together with those from other laboratories, resulted in a definition of the biological activity of the mucoproteins concerned in the isolation of the functional component and in the elucidation of its chemical structure.

The various observations, in their chronological order, leading to the information that certain mucoproteins display biological activity when influenza virus is used as indicator system, have been described in detail in a previous paper[15] in this Journal. The results up to that time and their interpretation may be summarized as follows:

1. Mucoproteins prepared from human urine (UM) and from bovine submaxillary glands (BSM) in an electrophoretically homogeneous state inhibit in high dilution haemagglutination by appropriately heat-treated

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virus particles (indicator virus) of certain strains. This holds also for the mucoprotein fraction ovomucin prepared from egg-white.[8]

2. When pretreated with living influenza virus or with a purified soluble enzyme (RDE) from vibrio cholerae, these mucoproteins lose irreversibly the ability to inhibit virus haemagglutination.[8]

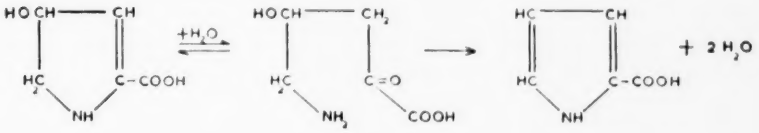
3. Concomitant with the loss of biological activity, a small molecular weight compound is released from the mucoprotein upon virus or RDE action.[23]

These observations indicated the operation of attractive forces between heated virus and the soluble mucoprotein resulting in adsorption of the virus particle to the fibrous mucoprotein and thus preventing haemagglutination. A further inference from the findings was the presence of an enzymatically active grouping at two or more sites of the virus surface attacking the inhibitory mucoprotein in such a way as to split off a small fragment of the molecule and, by doing so, to deprive the mucoprotein of its biological activity. Obviously, then, the biological activity of the mucoproteins towards the influenza virus resides in or is closely associated with the enzymatically liberated split product, and it seemed of great interest to find out the composition and structure of this segment of the mucoprotein.

As already mentioned in the earlier review,[15] the split product exhibited quite unusual features. It had reducing power and showed several reactions characteristic for carbohydrates, but decomposed with humin formation when treated with 1.0 N HCl for 30 minutes at 100° C. It contained nitrogen and after treatment with 0.1 N sodium carbonate for 12 minutes at 100° C. it coupled with the Ehrlich reagent (p-dimethylaminobenzaldehyde in hydrochloric acid) to give a purple color which spectrophotometrically was identical with that produced by an authentic specimen of N-acetyl D-glucosamine. In contrast to N-acetylglucosamine, however, the chromogen was alkali stable and after treatment with 1.0 N HCl at 100° for 60 minutes it failed to give the reaction of Elson and Morgan[9] shown by N-acetylhexosamine after acid de-acetylation. Odin,[22] confirming our results, added that the split product gave the Ehrlich reaction even without pretreatment with alkali (direct Ehrlich reaction) and drew attention to the identity, in their general reactions, of the split product and of sialic acid, a reducing acid of the probable composition $C_{13}H_{23}O_{11}N$ or $C_{13}H_{21}O_{10}N \cdot H_2O$ but of unknown structure,[3] first isolated by Blix[4] from bovine submaxillary gland mucin.

A major step towards the elucidation of the structural chemistry of the enzymatically released compound was taken when 2-carboxy-pyrrole was isolated[14, 17] from the alkali hydrolysate of homogeneous inhibitory mucoproteins (UM and BSM). The 2-carboxy-pyrrole, like the split product,

reacts with Ehrlich reagent directly, i.e., without alkali pretreatment and, being unstable towards mineral acid, loses its chromogenic quality on treatment with acid. Our assumption that 2-carboxy-pyrrole was present as such in the inhibitory mucoproteins and in the split product was not borne out by further work. The high value of the molecular extinction coefficient of 2-carboxy-pyrrole ($\epsilon = 11700$ in phosphate buffer, pH7.0) at a wavelength ($\lambda_{max} = 256$ m$\mu$), where the aromatic amino acids have a low extinction coefficient, would have rendered it possible to detect any preformed 2-carboxy-pyrrole in the native mucoprotein and in the split product. Contrary to expectation neither the undegraded mucoprotein nor the untreated split product exhibited absorption at 256 m$\mu$. However, when the split product was treated with 0.1 N sodium carbonate for 20 minutes at 100°, a substance could be extracted with ether from the acidified solu-

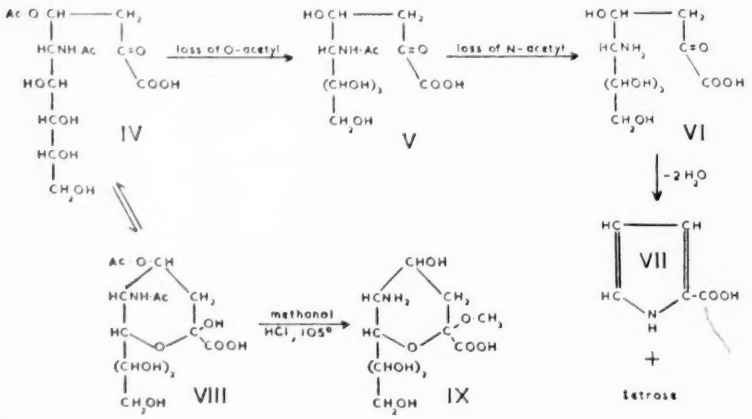



I II III

tion which on spectrophotometric and chromatographic analysis proved to be identical with synthetic 2-carboxy-pyrrole.[16, 17] These findings suggested the presence in the mucoprotein and in the split product of a structure readily converted to 2-carboxy-pyrrole under very mild alkaline conditions. Since glutamine, glutamic acid, 2-carboxy-5-pyrrolidone, proline, hydroxyproline, N-acetylglucosamine under mild alkaline conditions did not yield 2-carboxy-pyrrole, it was postulated[6] that the immediate precursor of 2-carboxy-pyrrole is the unstable 2-carboxy-4-hydroxypyrroline (I) which in aqueous solution will undergo reversible ring-opening. The open-chain product (II) as a $\beta$-oxyketone would be unstable to alkali (or acid) and reclosure of the ring will result in the formation of 2-carboxy-pyrrole (III). It was further assumed[6, 17] that within the framework of the native mucoprotein, and in the split product, I is prevented from spontaneous rearrangement to III by substitution at the hydroxyl group (R for H). Removal by alkali or acid of the substituent R will induce production of III.

Recent developments have both substantiated and qualified this concept. The coincidence in their general properties of sialic acid from BSM and of the split product from BSM and UM and our further observation[18] that along with the enzymatic release of the split product the sialic acid content

of BSM was reduced by 64 p.c., as estimated spectrophotometrically, suggested close structural relationship, if not identity, of the two compounds and, by implication, marked sialic acid as the component of BSM giving rise to the formation of 2-carboxy-pyrrole upon alkali treatment of this inhibitor. Blix, Lindberg, Odin, and Werner[2] found sialic acid prepared from BSM to contain an N-acetyl group, an O-acetyl group, which is very easily split off, a primary alcohol group, an $\alpha$-hydroxy group, and a total of five hydroxyl groups. On the basis of these data, its empirical formula $C_{13}H_{21}O_{10}N \cdot H_2O$, its general properties, and its convertability to 2-




carboxy-pyrrole we have assigned[18] to sialic acid the provisional structure IV. Obviously, IV by loss of its two acetyl groups would form VI, i.e., a delta-substituted H, which in turn would cyclise to the pyrrole VII with elimination of a tetrose. The susceptibility to acid and alkali of the ester and amide linkages joining the two acetyl groups to the rest of the sialic acid molecule would account for the formation of 2-carboxy-pyrrole and for the direct Ehrlich reaction given by sialic acid and the split product.

An important contribution bearing on the composition of the split product was made by Klenk and collaborators. In 1951 Klenk[24] isolated from gangliosides the methoxy derivative of neuraminic acid, and recently the same compound was prepared from bovine submaxillary gland mucin and from the inhibitory urine mucoprotein.[25] This compound has no reducing power, is ninhydrin positive, and is stable to alkali; therefore, it does not give the Ehrlich reaction in the cold after pretreatment with alkali. However, when heated in mineral acid, the methoxy derivative of neuraminic acid resembles sialic acid and the split product in the colours formed

with p-dimethylaminobenzaldehyde and with orcinol and in the intense discoloration on heating even with dilute acid. The compound, on elementary analysis, was given the formula $C_{11}H_{21}O_9N$; it does not contain acetyl groups. Recently Klenk and Faillard[28a] isolated N-acetyl neuraminic acid, $C_{12}H_{21}O_{10}N$, from bovine submaxillary gland mucoprotein. This acid exhibits the same general properties as sialic acid; unlike the methoxy derivative it has reducing power, is ninhydrin negative, and, most important, produces 2-carboxy-pyrrole on mild alkali treatment. Finally, it was shown by Klenk, Faillard, and Lampfrid[27] that the enzymatically released split

TABLE 1.

| | C | H | N |
|---|---|---|---|
| N-Acetyl neuraminic acid | | | |
| (a) Calculated for $C_{12}H_{21}O_{10}N$ | 42.48 | 6.23 | 4.13 |
| (b) Calculated for $C_{11}H_{19}O_9N$ | 42.72 (+0.6%)* | 6.15 (−1.3%) | 4.53 (+9.7%) |
| Found | 42.43, 42.17 (0.6%)† | 6.27, 6.32 (0.8%) | 4.07, 4.11, 4.11 (1.7%) |
| Methoxy neuraminic acid | | | |
| (a) Calculated for $C_{11}H_{21}O_9N$ | 42.44 | 6.80 | 4.50 |
| (b) Calculated for $C_{10}H_{19}O_9N$ | 42.70 (+0.6%)* | 6.76 (−0.6%) | 4.98 (+10.7%) |
| Found | {42.26, 42.48 / 42.51, 42.65} (0.9%)† | {6.84, 6.97 / 7.03, 7.10} (3.8%) | {4.31, 4.43 / 4.51, 4.73} (9.7%) |

* Denotes increase (+) or decrease (−) of b with respect to a.
† Denotes difference between extreme values.

product from UM on purification is identical with N-acetyl neuraminic acid. In our opinion the relationship between sialic acid, N-acetyl neuraminic acid, and 2-carboxy-pyrrole on the one hand and between sialic acid and the methoxy derivative of neuraminic acid on the other hand is best expressed by the structures IV-IX.

This scheme requires a comment. Whereas Klenk and associates[28a, 27, 28] consider N-acetyl neuraminic acid and the methoxy derivative of neuraminic acid to have the formulae $C_{12}H_{21}O_{10}N$ and $C_{11}H_{21}O_9N$, respectively, we believe that their analytical data are not at variance with the formulae $C_{11}H_{19}O_9N$ and $C_{10}H_{19}O_9N$, respectively, as assigned to the two compounds in V and IX. In Table 1 the calculated values for the elements according to the composition assigned to them by Klenk and assigned to them in V and IX are compared with the data published by Klenk and associates.[28a, 28b, 27, 28] As may be seen from the figures for methoxy neuraminic acid, the differences between the C, H, and N values for the two assumed formulae are not larger than the differences between two individual analyses

of these elements, i.e., they fall within the experimental error of the method. The same holds for the C and H values of N-acetyl neuraminic acid. The analytical figures for N of N-acetyl neuraminic acid, taken at their face value, seem to fit Klenk's formula better than V. An examination, however, of the experimental details provided by Klenk shows that due to the very small amount of material (2.04-2.40 mg.) available for the Kjeldahl procedure a difference of 0.04 ml. (one drop) of N/100 HCl which is within the error of the method introduces an experiment error of ± 7 p.c. under these conditions. We conclude from the above analysis of Klenk's figures that they do not allow a differentiation between the formulae as proposed by him and as suggested in V and IX, respectively; they are consistent with our interpretation.

From Blix's data[2] it seems very probable that sialic acid from BSM is diacetyl neuraminic acid (IV) and it may be useful to restrict this name to the diacetyl derivative* of neuraminic acid. The details of Blix's latest procedure have not yet been published; there remains the uncertainty that different preparations of bovine sialic acid though exhibiting the same X-ray diagrams are not consistent enough in their elementary analysis to establish definitely the composition of BSM sialic acid.[2] Should the presence of an O-acetyl group in this compound be confirmed, then N-acetyl neuraminic acid (V) as obtained from BSM by Klenk[26a] is an artifact derived from sialic acid (IV) by loss of the O-acetyl group during purification. The same may be true for the split product released by the viral enzyme from UM.[27] Blix and associates[2] have prepared from ovomucin "ovine sialic acid" of the composition $C_{11}H_{19}O_9N$ containing an N-acetyl group only and almost certainly identical with N-acetyl neuraminic acid. "Porcine sialic acid" has no acetyl group but instead an N-glycolyl group.[2]

It would be most desirable to establish firmly whether or not the N-substituted neuraminic acids of bovine and porcine submaxillary gland mucins, of UM, and of ovomucin have an O-acetyl group in addition to the N-acyl group, because this question has wider implications. N-Acetyl neuraminic acid in solution is unstable; it decomposes under discoloration within days even at 1° C.[27] If, as Klenk[26a, 27] assumes, N-acetyl neuraminic acid is present as such in BSM and UM, it must be stabilized by its linkage to the adjacent unit within the mucoprotein molecule; for an 1 p.c. dispersion of ovomucin containing 0.04 p.c. lysozyme (final concentration) may be kept at 0° C. for months without losing its biological activity or changing its snow-white colour. Stabilization of V other than by O-acetyl (IV)

* To Blix[1] goes the credit of having first isolated this compound from BSM. It seems, however, inadvisable to denote with the same name compounds of different chemical composition though they may be closely related to or derived from sialic acid.

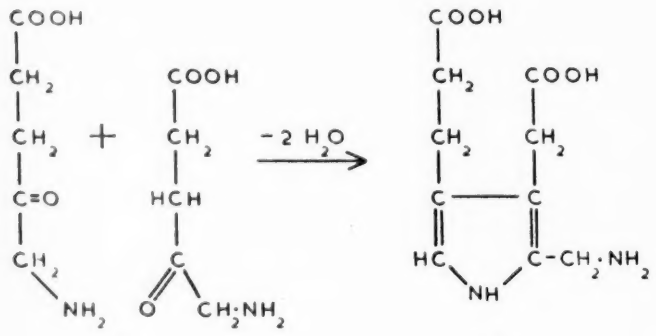
would result from the engagement of its keto group in a glycosidic linkage in analogy to IX. An ordinary O-glycosidic linkage may be excluded from the facts that both the native mucoprotein and N-acetyl neuraminic acid form 2-carboxy-pyrrole on alkali treatment,[14, 28a] whereas the acetal or O-glycoside methoxy neuraminic acid does not (10N NaOH, 2 hr., 100° C.).[28a] An N-glycosidic linkage mediated by the amino group of an adjacent amino acid or amino sugar would stabilize V efficiently and would explain the release of sialic acid from BSM by the simple expedient of heating its solution at neutrality to 110-115° C.;[1] for many synthetic N-glycosides are known to hydrolyse under very mild conditions, some of them even at pH 7.0.[14, 29, 33] Such a linkage would in no way hinder the formation of 2-carboxy-pyrrole from mucoproteins on alkali treatment; moreover, its cleavage by the action of RDE, involving the loss of a $-COO^-$ group and the gain of a $-NH_3^+$ group, would account for the considerable reduction in electrophoretic mobility of BSM and UM after such treatment.[7, 28] In this context it is of interest that Inouye and Onodera[23] and Dische and Jacubeit[9] have isolated from glycoproteins compounds consisting of a mono- or disaccharide in N-glycosidic linkage with the amino group of an amino acid.

Though the scheme presented seemed to be consistent with the data provided by the Swedish, German, and Australian laboratories, it was desirable to obtain further experimental evidence for its validity. The structure assigned to neuraminic acid (VI) may be visualized as an aldol type of condensation product of 2-amino 2-deoxyhexose with pyruvic acid, a type of linkage rendering recovery of the amino sugar by acid treatment impracticable and yet allowing the masked hexosamine to play its full part in the chromogen formation in the direct and indirect Ehrlich reactions, as was indeed observed.[3, 12, 25] The tendency of pyruvic acid to condense with aldehydes has long been known; thus the reaction

$$C_6H_5 \cdot CHO + CH_3 \cdot CO \cdot COOH \xrightarrow{H_2O} C_6H_5 \cdot CH : CH \cdot CO \cdot COOH$$

proceeds at 5° C. when carried out in the presence of NaOH (Erlenmeyer[10]). On this reasoning it was expected that under alkaline conditions pyruvic acid would condense with hexosamine to form VI, a reaction accompanied by closure of the ring, loss of 2 moles water, rearrangement of the double bonds and elimination of a tetrose (VI→VII). This was verified experimentally.[28] When pyruvic acid (0.1 N solution) was allowed to react with D-glucosamine in the molecular ratio 1 : 3 at 100° for several hours under controlled conditions of alkalinity, 2-carboxy-pyrrole could be extracted from the reaction mixture in a yield of about 20 p.c.; the compound was obtained in crystalline form and identified so far by its melting

point, mixed melting point, U-V absorption spectrum, and chromatographic behaviour.[9] D-Galactosamine was found to be as suitable a partner in the condensation reaction as D-glucosamine, both yielding 2-carboxy-pyrrole.[9] The condensation of hexosamine and pyruvic acid to the pyrrole

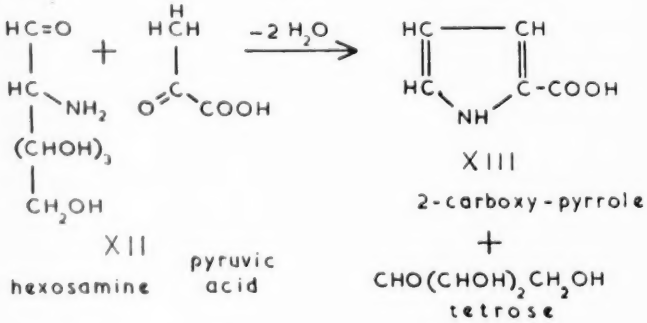



X

δ-aminolaevulinic acid

XI

porphobilinogen




XII

hexosamine

pyruvic acid

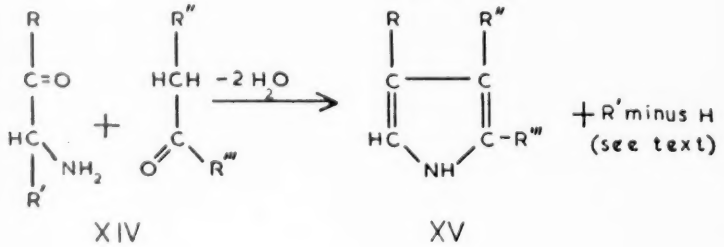
XIII

2-carboxy-pyrrole

+

$CHO(CHOH)_2CH_2OH$

tetrose




XIV

XV

compound may be regarded as an analogue to the Knorr type condensation of 2 mols of δ-amino-laevulinic acid to the pyrrole derivative porphobilinogen (X-XIII), demonstrated by Shemin and Russell[45] and by Neuberger and Scott.[41] In both cases the same reactive groups are involved in the condensation (XIV and XV). The main difference is that in δ-aminolaevulinic acid R' = H and on condensation 2 mols $H_2O$ are formed concomitant with pyrrole synthesis; in hexosamine R' = $CHOH \cdot (CHOH)_2 \cdot CH_2OH$ and upon condensation with pyruvic acid fragmentation of the hexosamine structure by reverse aldolization takes place resulting in the production of 2-carboxy pyrrole, 2 mols $H_2O$ and a tetrose; the latter is further degraded under the prevailing alkaline conditions.

Neuraminic acid (VI), being an α-keto acid, is readily decarboxylated by mineral acid to form the next lower aldehyde. This aldehyde may be regarded as a substituted 2-deoxyhexose and it is due to this structural feature that sialic acid gives a very similar colour reaction (violet colour with maximum absorption at 530 mμ) with Dische's diphenylamine reagent as do 2-deoxyhexoses[18] and glycals (red colour with maximum absorption 515 mμ). Quite probably the same structural feature of 2-deoxy-sugar is responsible for the decomposition with discoloration of sialic acid and N-acetyl neuraminic acid on heating with dilute mineral acid. 2-deoxy-glucose,[28] and 2-deoxy-galactose* exhibiting the same property. It is thus evident that all properties described for sialic acid, N-acetyl neuraminic acid and the split product are consistent with structures IV and V; the exact position of the O-acetyl group in IV remains to be determined.

From the previous discussion it is obvious that the direct Ehrlich reaction as given by BSM, UM and ovomucin is due to the presence in them of N-acetyl neuraminic acid or of diacetyl neuraminic acid (sialic acid). There is no indication that these or similar mucoproteins contain any other component but neuraminic acid reacting after rearrangement directly, i.e. without alkali pretreatment, with Ehrlich reagent to give a stable colour with absorption maximum at 565 mμ under standard conditions as defined by Werner and Odin.[46] This statement is based on two series of observations: (1) The following substances failed to give a positive direct Ehrlich reaction as defined above: Hyaluronic acid, chondroitin sulfuric acid, heparin, carbohydrate free proteins, tryptophan.[47] 2. The determination of "sialic acid" in a variety of mucoproteins by independent methods (diphenylamine reaction, tryptophan perchloric acid reaction) gave values varying by not more than 10 p.c. from those obtained by the method based on the direct Ehrlich reaction.[20] It seems therefore legitimate to apply the

* Dr. E. L. Hess, Chicago, personal communication.

direct Ehrlich reaction as an approximate measure of the neuraminic acid content of a mucoprotein though a check by an independent method may be desirable. The value obtained is approximate only since it is customary to take Blix's sialic acid preparation from BSM as standard and we cannot be absolutely sure that the various neuraminic acid derivatives (N-acetyl neuraminic acid, N-glycolyl neuraminic acid, and possibly others) give molecule for molecule under standard conditions the same colour intensity as sialic acid. With this reservation Table 2 summarizes the amounts in some mucoproteins of direct Ehrlich-reacting neuraminic acid derivatives expressed as sialic acid.

TABLE 2. "SIALIC ACID" CONTENT* OF VARIOUS MUCOPROTEINS

| *Mucoprotein* | *Sialic acid p.c.* | *References* |
|---|---|---|
| Bovine submax. gland mucoprotein | 16.8 | 37 |
| Bovine serum albumin | 1.7† | 21 |
| Acid glycoprotein of human plasma | 10.1 | 37 |
| Seromucoid (Rimington) | 8.5 | 37 |
| $\alpha_2$-Globulin (Cohn's fraction IV-6-2) | 5.7 | 37 |
| Human urine inhibitory mucoprotein | 9.2 | 32 |
| Human meconium | 8.7† | 21 |
| Blood-group active mucoids from ovarian cyst fluids | 1.1-8.2 | 11 |
| Thyroglobulin | 2.7† | 21 |
| Ovomucin | 5.8 | 37 |
| Ovomucoid | 2.0† | 21 |

* See text.
† Standard = homogeneous bovine submax. gland mucoprotein assuming a content of 16.8 p.c. sialic acid.

In contrast to the direct Ehrlich reaction a positive Ehrlich reaction of a mucoprotein after mild alkali treatment (0.05 N $Na_2CO_3$, 15 minutes, 100° C.), first observed by Morgan and King,[20] does not lend itself to a ready interpretation. This indirect Ehrlich reaction is a complex reaction involving neuraminic acid, N-acetylglucosamine, N-acetylgalactosamine, N-glycosyl amino acids and possibly other substances. The mechanism of this reaction is not fully understood due to the variety of reactions sugars are undergoing under the impact of alkali (enolization, condensation, fragmentation, recombination, disproportionation, etc.[38]). Several chromogens are formed in the reaction. Stanley,[39] participating in our earlier work, has separated by dialysis material liberated by 0.05 N $Na_2CO_3$ (20 minutes, 100° C.) from ovomucin and demonstrated chromatographically the

presence of four different substances reacting after this treatment with Ehrlich reagent; in addition to these chromogens, unchanged free N-acetylglucosamine was found to be present. We have submitted BSM to the same mild alkali treatment for 15 minutes and detected in the concentrated dialysate three different chromogens by chromatography (solvent: n-butanol / pyridine / water; $R_F$ values 0.15, 0.80, 0.90) reacting with Ehrlich reagent. After acid hydrolysis of the dialysate an amino compound was isolated by adsorption to and elution from Dowex 50 according to Boas[4] and identified as galactosamine, the predominant amino sugar of BSM,[20] by oxidation with ninhydrin to lyxose. In this way 4 p.c. of the hexosamine known to be present in BSM was recovered unchanged. In view of these findings it seems rather arbitrary to compare the colour produced in the Ehrlich reaction of a mucoprotein after mild alkali treatment with an N-acetylglucosamine standard; until more is known about the chemistry and origin of the various chromogens formed and their colour intensity in the Ehrlich reaction compared with that given by N-acetylglucosamine, little information is conveyed by determining quantitatively the "N-acetylhexosamine colour"[11] of a mucoprotein.

From the evidence discussed in this paper it would appear that the biological activity of a group of mucoproteins, as displayed by their inhibitory effect upon haemagglutination by heated influenza virus, resides in the N-acetyl neuraminic acid component of these conjugated proteins; this has been established for BSM, UM, and ovomucin. Whether or not one or more of them contain in addition an O-acetyl group has yet to be proven; for BSM the presence of neuraminic acid in its diacetyl form seems extremely probable. Neuraminic acid has been shown with a high degree of probability to be an aldol type of condensation product of hexosamine with pyruvic acid. The release of N-acetyl neuraminic acid, catalysed by the influenza virus enzyme and by a soluble enzyme from vibrio cholerae, irreversibly inactivates the mucoprotein. N-Acetyl neuraminic acid represents, therefore, the functional group of the virus haemagglutinin inhibitory mucoproteins, either on its own or as the specific component of a terminal unit.

Klenk, Faillard, and Lempfrid[27] have confirmed our previous observation[22] that paper chromatography of the split product reveals the presence of several amino acids. However, the authors apparently have not yet tested the controls for these ninhydrin reacting substances; the matter is still under investigation. In our experience BSM, UM, and ovomucin release, for some unknown reason, continuously minute amounts of amino acids; the chromatographic analysis of the split product has, therefore, carefully to be checked against the chromatograms obtained from appropriate con-

trols. If this is done, possibly one or two amino acids remain, and their release may be associated with the virus-mucoprotein interaction.[14]

It is to be hoped that continued research will consolidate our knowledge about the chemistry of the various neuraminic acid derivatives present in mucoproteins and lead to the chemical and enzymatic synthesis of N-acetyl neuraminic acid. From such studies there might eventually emerge a clearer concept of the function of neuraminic acid which seems to represent a unique example of a fusion product between a static and a dynamic sugar derivative.

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# EMERGENCE OF PERMANENT FIRST MOLARS IN THE MONKEY (*MACACA MULATTA*). ASSOCIATION WITH OTHER GROWTH PHENOMENA‡§

## INTRODUCTION

The main purpose of the present report is to examine emergence data on the permanent first molars of the macaque against the background of similar data on the entire deciduous dentition. In addition to this an attempt will be made to link the phenomenon of odontiasis with other expressions of growth and development in the same primate. The ultimate objective of these investigations is to obtain clues which might be of value in designing better studies of the human dentition in the future.

Very little is actually known about the subject matter with which this paper deals. Over one hundred years ago Youatt[24] found the inspection of the dentition useful in estimating the ages of calves and cows, and soon thereafter Saunders[22] exhorted members of the English Parliament to use the teeth as a criterion of age to remove certain difficulties in the application of the child labor law.

The ranges of variation normally encountered in dealing with erupting teeth are quite wide. Until the variations and correlations occurring normally in the course of development are known, there is no satisfactory frame of reference for interpreting observations on odontiasis in experimental animals. Some of the problems that confront the investigator who wishes to construct reasonably reliable statistical standards for estimating the chronological age of an infant macaque are discussed at length in a previous paper by the authors.[18] The present report can be looked upon as a continuation and expansion of the aforementioned study.

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TABLE 1. AGES OF EMERGENCE OF ALL DECIDUOUS TEETH AND OF THE PERMANENT FIRST MOLARS IN 26 TO 27 MALE MACAQUES
Figures for deciduous teeth represent averages for a tooth and its antimere. The data are in terms of years after birth.

| *Number of animal* | *Maxilla* | | | | | | | *Mandible* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $M_1$ | | | | | | | $M_1$ | |
| | $di_1$ | $di_2$ | $dc$ | $dm_1$ | $dm_2$ | *Rt.* | *Lt.* | $di_1$ | $di_2$ | $dc$ | $dm_1$ | $dm_2$ | *Rt.* | *Lt.* |
| 603 | .01 | .03 | .10 | .13 | .30 | 1.19 | 1.17 | .01 | .01 | .10 | .13 | .29 | 1.08 | 1.04 |
| 728 | .00 | .05 | .16 | .16 | .34 | 1.35 | 1.35 | .01 | .01 | .16 | .16 | .30 | 1.23 | 1.23 |
| 642 | .01 | .06 | .13 | .16 | .40 | 1.40 | 1.40 | .01 | .01 | .13 | .16 | .40 | 1.28 | 1.32 |
| 590 | .04 | .10 | .13 | .13 | .39 | 1.33 | 1.31 | .02 | .05 | .13 | .14 | .39 | 1.18 | 1.18 |
| 719 | .03 | .10 | .12 | .16 | .37 | 1.37 | 1.37 | .03 | .07 | .15 | .16 | .33 | 1.30 | 1.30 |
| 712 | .05 | .09 | .16 | .16 | .40 | 1.30 | 1.30 | .03 | .05 | .15 | .17 | .33 | 1.16 | 1.16 |
| 625 | .03 | .09 | .18 | .18 | .41 | 1.48 | 1.42 | .03 | .04 | .16 | .19 | .43 | 1.32 | 1.32 |
| 484 | .00 | .09 | .17 | .20 | .40 | 1.47 | 1.47 | .00 | .01 | .20 | .25 | .39 | 1.41 | 1.41 |
| 510 | .07 | .09 | .21 | .21 | .36 | 1.32 | 1.32 | .02 | .05 | .22 | .21 | .36 | 1.20 | 1.17 |
| 665 | .04 | .09 | .16 | .20 | .44 | 1.54 | 1.54 | .03 | .06 | .18 | .20 | .40 | 1.29 | 1.36 |
| 288 | .06 | .09 | .17 | .13 | .48 | 1.52 | 1.52 | .05 | .07 | .20 | .15 | .43 | 1.42 | 1.44 |
| 612 | .03 | .11 | .16 | .21 | .43 | 1.49 | 1.49 | .07 | .07 | .16 | .20 | .42 | 1.37 | 1.37 |
| 449 | .04 | .07 | .15 | .17 | .40 | 1.92 | 1.90 | .01 | .04 | .17 | .21 | .40 | 1.90 | 1.90 |
| 709 | .07 | .13 | .25 | .19 | .39 | 1.28 | 1.28 | .05 | .08 | .26 | .23 | .42 | 1.26 | 1.26 |
| 717 | .07 | .15 | .20 | .21 | .46 | 1.63 | 1.63 | .05 | .08 | .. | .20 | .41 | 1.38 | 1.38 |
| 521 | .06 | .12 | .19 | .21 | .49 | 1.51 | 1.51 | .06 | .12 | .19 | .21 | .41 | 1.43 | 1.43 |
| 718 | .08 | .13 | .22 | .18 | .49 | 1.61 | 1.71 | .07 | .07 | .19 | .17 | .43 | 1.54 | 1.54 |
| 525 | .07 | .11 | .20 | .22 | .45 | 1.53 | 1.53 | .07 | .08 | .21 | .24 | .40 | 1.38 | 1.38 |
| 553 | .10 | .13 | .21 | .19 | .50 | 1.42 | 1.52 | .07 | .07 | .22 | .19 | .50 | 1.27 | 1.27 |
| 556 | .08 | .13 | .22 | .22 | .46 | 1.47 | 1.47 | .05 | .09 | .22 | .22 | .46 | 1.36 | 1.36 |
| 477 | .07 | .10 | .23 | .23 | .50 | 1.67 | 1.71 | .06 | .07 | .17 | .23 | .44 | 1.55 | 1.60 |
| 280 | .07 | .14 | .18 | .20 | .56 | 1.79 | 1.83 | .10 | .10 | .20 | .25 | .46 | 1.52 | 1.56 |
| 440 | .05 | .15 | .20 | .28 | .58 | 1.63 | 1.63 | .05 | .05 | .20 | .30 | .52 | 1.63 | 1.55 |
| 660 | .09 | .15 | .26 | .28 | .54 | 1.62 | 1.71 | .06 | .09 | .23 | .26 | .54 | 1.47 | 1.47 |
| 573 | .10 | .15 | .30 | .28 | .60 | ... | ... | .08 | .10 | .30 | .28 | .52 | 1.58 | 1.56 |
| 686 | .12 | .18 | .31 | .26 | .54 | 1.61 | 1.61 | .12 | .14 | .35 | .27 | .51 | 1.36 | 1.36 |
| 483 | .15 | .23 | .28 | .30 | .57 | 1.71 | 1.71 | .09 | .16 | .30 | .28 | .48 | 1.51 | 1.51 |

## MATERIAL AND METHODS

The material studied consists of the dental records of 27 male and 42 female macaques born and reared in the monkey colony of the Department of Obstetrics and Gynecology at the Yale University School of Medicine. Only records on presumably normal animals were included, as it is known that certain pathological conditions, such as rickets[7, 10, 27, 36] and certain types of hormonal interference, for example, treatment with testosterone propionate,[30] may affect tooth eruption. As the dental effects of castration are not known with certainty, the records on several castrated males were excluded also. A pseudohermaphrodite female likewise was left out of the series.

TABLE 2. AGES OF EMERGENCE OF ALL DECIDUOUS TEETH AND OF THE PERMANENT FIRST MOLARS IN 40 TO 42 FEMALE MACAQUES
Figures for deciduous teeth represent averages for a tooth and its antimere. The data are in terms of years after birth.

| Number of animal | Maxilla | | | | | | | Mandible | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $M_1$ | | | | | | | $M_1$ | |
| | $di_1$ | $di_2$ | $dc$ | $dm_1$ | $dm_2$ | Rt. | Lt. | $di_1$ | $di_2$ | $dc$ | $dm_1$ | $dm_2$ | Rt. | Lt. |
| 582 | .03 | .04 | .11 | .14 | .38 | 1.40 | 1.40 | .00 | .00 | .11 | .14 | .34 | 1.26 | 1.25 |
| 404 | .01 | .09 | .15 | .15 | .33 | 1.46 | 1.46 | .01 | .04 | .15 | .15 | .31 | 1.19 | 1.15 |
| 591 | .03 | .09 | .15 | .15 | .31 | 1.28 | 1.32 | .03 | .04 | .14 | .16 | .33 | 1.15 | 1.21 |
| 615 | .03 | .08 | .17 | .13 | .32 | 1.35 | 1.35 | .02 | .03 | .18 | .16 | .32 | 1.20 | 1.18 |
| 442 | .03 | .08 | .17 | .16 | .34 | 1.28 | 1.35 | .04 | .06 | .14 | .14 | .29 | 1.24 | 1.24 |
| 641 | .01 | .07 | .17 | .15 | .38 | 1.32 | 1.30 | .02 | .04 | .18 | .17 | .34 | 1.27 | 1.23 |
| 730 | .02 | .08<.16 | | <.16 | .44 | 1.35 | 1.35 | .01 | .03<.16 | | .18 | .40 | 1.23 | 1.23 |
| 441 | .04 | .14 | .15 | .14 | .34 | 1.39 | 1.33 | .04 | .06 | .15 | .16 | .32 | 1.25 | 1.23 |
| 705 | .04 | .08 | .17 | .12 | .41 | 1.27 | 1.29 | .03 | .05 | .19 | .16 | .41 | 1.27 | 1.27 |
| 617 | .05 | .09 | .16 | .16 | .42 | 1.32 | 1.32 | .06 | .07 | .16 | .15 | .32 | 1.22 | 1.22 |
| 485 | .03 | .11 | .20 | .14 | .39 | 1.42 | 1.40 | .03 | .09 | .20 | .20 | .29 | 1.24 | 1.24 |
| 589 | .06 | .09 | .17 | .17 | .38 | 1.36 | 1.36 | .05 | .05 | .19 | .19 | .36 | 1.21 | 1.32 |
| 651 | .07 | .10 | .18 | .20 | .43 | 1.34 | 1.41 | .01 | .03 | .19 | .19 | .37 | 1.34 | 1.34 |
| 272 | .06 | .09 | .17 | .18 | .43 | 1.32 | 1.32 | .04 | .07 | .18 | .19 | .41 | 1.24 | 1.20 |
| 482 | .04 | .10 | .17 | .17 | .42 | 1.50 | 1.50 | .02 | .04 | .17 | .21 | .35 | 1.37 | 1.37 |
| 568 | .03 | .10 | .17 | .17 | .41 | 1.29 | 1.40 | .04 | .04 | .17 | .27 | .41 | 1.27 | 1.27 |
| 474 | .03 | .08 | .20 | .20 | .41 | 1.39 | 1.41 | .03 | .04 | .23 | .23 | .41 | 1.32 | 1.32 |
| 289 | .04 | .12 | .21 | .19 | .48 | 1.49 | 1.47 | .04 | .07 | .22 | .19 | .32 | 1.37 | 1.37 |
| 518 | .05 | .11 | .20 | .19 | .41 | 1.47 | 1.49 | .03 | .06 | .26 | .20 | .38 | 1.34 | 1.34 |
| 638 | .09 | .14 | .18 | .16 | .48 | 1.29 | 1.29 | .02 | .10 | .18 | .19 | .44 | 1.39 | 1.27 |
| 656 | .07 | .13 | .22 | .15 | .42 | 1.37 | 1.37 | .06 | .07 | .22 | .20 | .38 | 1.26 | 1.26 |
| 659 | .08 | .11 | .20 | .18 | .40 | 1.37 | 1.37 | .06 | .08 | .20 | .21 | .41 | 1.21 | 1.21 |
| 520 | .05 | .10 | .23 | .21 | .42 | 1.53 | 1.49 | .05 | .06 | .23 | .21 | .40 | 1.31 | 1.31 |
| 563 | .07 | .14 | .21 | .16 | .37 | 1.32 | 1.32 | .09 | .09 | .21 | .21 | .37 | 1.32 | 1.32 |
| 285 | .05 | .07 | .21 | .19 | .57 | 1.52 | 1.56 | .06 | .07 | .18 | .18 | .47 | 1.39 | 1.43 |
| 291 | .06 | .11 | .20 | .18 | .49 | 1.58 | 1.56 | .05 | .07 | .19 | .21 | .44 | 1.41 | 1.44 |
| 736 | .08 | .11 | .25 | .19 | .44 | 1.38 | 1.38 | .06 | .07 | .26 | .22 | .40 | 1.29 | 1.29 |
| 292 | .06 | .11 | .20 | .20 | .51 | 1.54 | 1.54 | .05 | .08 | .20 | .20 | .45 | 1.37 | 1.37 |
| 507 | .. | .. | .. | .. | .48 | 1.49 | 1.49 | .. | .. | .. | .. | .46 | 1.37 | 1.37 |
| 652 | .07 | .13 | .23 | .20 | .44 | 1.46 | 1.56 | .05 | .07 | .23 | .21 | .44 | 1.46 | 1.38 |
| 731 | .09 | .14 | .21 | .23 | .46 | 1.49 | 1.49 | .06 | .09 | .22 | .22 | .39 | 1.22 | 1.29 |
| 567 | .09 | .12 | .21 | .27 | .44 | 1.42 | 1.44 | .06 | .08 | .21 | .27 | .40 | 1.31 | 1.31 |
| 456 | .06 | .15 | .23 | .25 | .47 | 1.62 | 1.60 | .04 | .08 | .25 | .25 | .43 | 1.56 | 1.56 |
| 451 | .05 | .15 | .25 | .25 | .52 | 1.65 | 1.67 | .05 | .07 | .28 | .22 | .44 | 1.48 | 1.48 |
| 452 | .04 | .12 | .31 | .21 | .52 | 1.56 | 1.56 | .06 | .07 | .27 | .23 | .51 | 1.50 | 1.50 |
| 527 | .. | .. | .. | .. | .48 | 1.66 | 1.66 | .. | .. | .. | .. | .. | 1.45 | 1.40 |
| 470 | .09 | .14 | .29 | .19 | .55 | 1.54 | 1.56 | .07 | .09 | .29 | .19 | .49 | 1.40 | 1.40 |
| 733 | .09 | .12 | .27 | .25 | .51 | 1.55 | 1.63 | .06 | .10 | .29 | .23 | .43 | 1.41 | 1.41 |
| 448 | .08 | .14 | .28 | .25 | .55 | 1.64 | 1.64 | .08 | .08 | .32 | .25 | .37 | 1.33 | 1.37 |
| 580 | .10 | .18 | .26 | .22 | .45 | 1.53 | 1.55 | .10 | .12 | .26 | .24 | .43 | 1.39 | 1.38 |
| 732 | .09 | .15 | .29 | .26 | .52 | 1.55 | 1.55 | .09 | .15 | .30 | .26 | .44 | 1.41 | 1.41 |
| 447 | .12 | .16 | .22 | .25 | .55 | 1.61 | 1.61 | .10 | .16 | .28 | .24 | .48 | 1.48 | 1.46 |

Since one of the purposes of the investigation was the establishment of statistical norms for the emergence of permanent first molars in the monkey, the selection of laboratory data was limited to the records which supplied information on these teeth, in addition to data on the deciduous dentition. The records of one male and two females without complete deciduous tooth data were added to the above material in order to obtain the largest possible number of individual items for the calculation of permanent first molar statistics. The basic first molar data will be found in Tables 1 and 2, in columns $M_1$.

Although seven generations are now represented in the colony, in which breeding for replacement was begun in 1935,[21] only about half of these contained animals that were old enough to supply data for the present study. The tooth population sample available may be regarded as a sufficiently random one, since the monkeys included in the series analyzed were members of a large number of genetically unrelated families.

The rôle of personal factors in making and recording the observations is negligible, considering that during the 20-year period under consideration all details pertaining to this phase of the investigation were attended to by one and the same individual.* For information on the procedural details followed in preparing the data for statistical analyses the reader is referred to the earlier work of the authors.[18] Only one exception need be noted, namely, that to insure the employment of the most probable ages of emergence in the calculations, 0.01 yr. was subtracted from laboratory entries based on weekly inspections and 0.04 yr. from those based on monthly examinations.

Some of the procedures followed in the analyses have been described elsewhere.[12, 18] Due to the fact that chronological asymmetry in the emergence of permanent teeth is not at all uncommon, the numerical values for a tooth and its antimere were never averaged, as was done in dealing with the deciduous dentition. It is worth emphasizing that the available data represent a random sampling from the universe of erupting teeth; they are the result of serial observations of such duration that no information has been excluded by commencing the study after some teeth already had emerged, or by terminating it before the completion of this process in some animals.

As the data obtained supply information on both ends of a range of distribution, cumulative percentages can be calculated for all age levels that appear in a series. After this the percentages can be plotted on ordinary arithmetically ruled graph paper. As a rule the arrangement of the plotted points is such that it is not difficult to draw a cumulative percentage ogive freehand in order to establish the line which furnishes the simplest graphic generalization of the location of the points. This line which is, in reality, a special type of regression curve, is drawn through as many individual points as possible; if difficulty is experienced in completing the ends of the line, recourse may be had to visualizing the universally occurring pattern that characterizes all graphic representations of similar data, based on a quantitatively adequate number of observations.

The freehand curve described above provides what appears to be a fair approximation to the hypothetical curve that would be obtained if an infinitely large population of

* All laboratory examinations were meticulously carried out by Mr. Joseph Negri, technical assistant. Prior to September, 1948, he made weekly inspections for emerging permanent first molars and monthly examinations after the date mentioned. The criterion of odontiasis was always the same, namely, the first appearance of a tooth crown, or any part thereof, above the oral mucosa.

these monkeys were available for study. The statistical expressions of variability that can be derived by suitable analyses of this curve have a high degree of stability and are surprisingly independent of the number of field or laboratory observations utilized for plotting the graphic scaffolding of the curve, provided, of course, that the basic data are not excessively limited in quantity.

The computations required for a mathematical description of chronological scattering in the emergence of permanent first molars can be based either directly on the values recorded in Tables 1 and 2, or indirectly on them by utilizing the freehand ogives mentioned above. Since the technique employed may be a matter of personal preference, the results obtained by the second technique were included in Table 3 for purposes of comparison.

The standard errors given in Table 3 were calculated by assuming that the two observations per animal were not independent, i.e., $N=1$ was considered established by the number of tooth pairs.

## RESULTS

A. *Emergence of permanent first molars and the influence of sex.* Some of the statistical data on the permanent first molars have been condensed into compact graphic form for more convenient presentation. Figures 1 to 4 present the authors' concept of the cumulative frequency curves that can be derived from the original data given in Tables 1 and 2, in columns $M_1$. The percentage values summarized by the smoothed curve are shown by small circles; the hypothesized extremes of the curve are indicated by small arrows.

Determination of the exact location of these terminals is extremely difficult in all cases and would require repeated examination of thousands of animals for the attainment of a high degree of reliability. The ends of the curves shown in Figures 1-4 are merely for the purpose of designating the most likely limits of distribution for the vast majority of normal animals. The points on the curves indicate where the latter are intersected by vertical lines that represent the limits of age intervals of 0.04 of a year; they may be helpful in translating the graphs into numerical values.

Estimates of the percentile distribution of emerging first molars may be made from the curves shown in Figures 1 to 4. It is evident that the degrees of asymmetry are not large; in all four instances a slight degree of skewing in the positive direction can be observed. It was not deemed necessary to make formal tests for normality.

The most important statistical values are shown in Table 3. In addition to the means, modes and medians were calculated also, but since they were only slightly below the first-mentioned measures of central tendency, they were not included in the table. As for the means and standard deviations, it

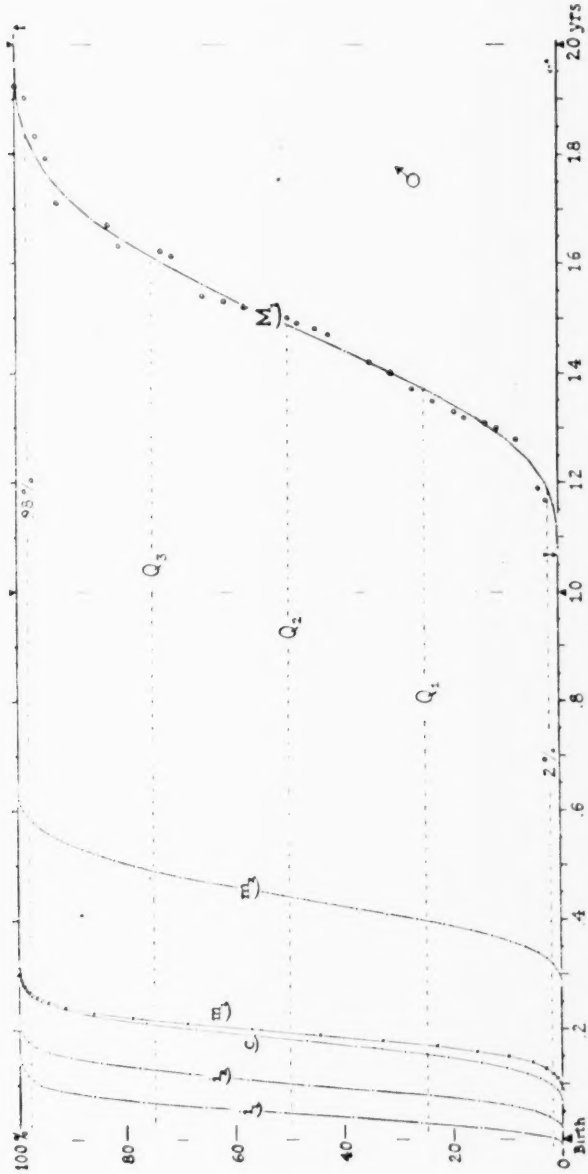


FIG. 1. Cumulative percentage ogives for the emergence of the permanent maxillary first molar ($M_1$) and the five classes of deciduous maxillary teeth of male macaques (*Macaca mulatta*).

The percentages obtained from original data are shown by small circles; the ends of the $M_1$ curve are indicated by arrows.

The points on deciduous teeth curves are 0.01 of a year apart; those on $M_1$ curve 0.04 of a year apart.

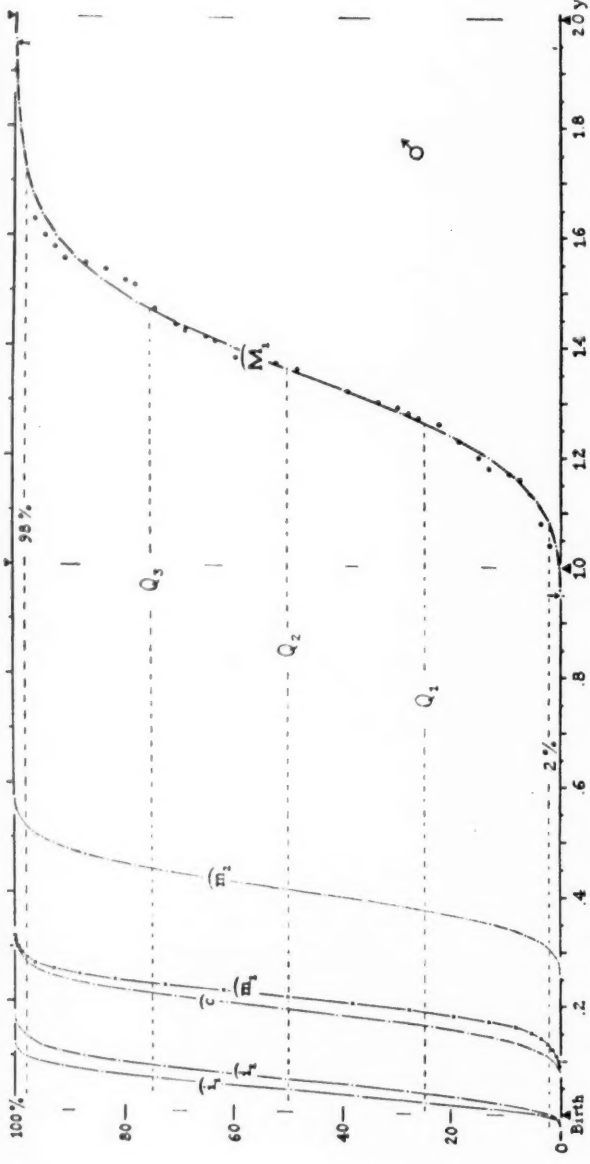


FIG. 2. Cumulative percentage ogives for the emergence of the permanent mandibular first molar ($\overline{M_1}$) and the five classes of deciduous mandibular teeth of male macaques (*Macaca mulatta*).

For explanation of details, see text under Figure 1.

is worthy of note that the two methods of calculation yield very similar results.

The data at hand indicate much less variability in the emergence of permanent first molars in female macaques than in males. This is a finding which, so far as the authors know, has not been reported before. It has no readily discoverable counterpart in man.[11]

Biometrically speaking there seems to be no doubt about the actuality of the sex difference just mentioned. The narrower range of variability is given by the data on the permanent first molars of females and not by those

TABLE 3. STATISTICAL SUMMARY OF AGES OF EMERGENCE OF PERMANENT FIRST MOLARS
Data expressed to the nearest hundredth of a year

| *Dental arch* | | *Number of tooth pairs* | *Computation based on* | | | | $S.E._{\bar{M}}$ |
|---|---|---|---|---|---|---|---|
| | | | *Original scores* | | *Smoothed ogive* | | |
| | | | *Mean* | $\sigma$ | *Mean* | $\sigma$ | |
| Males | Max. | 26 | 1.51 | 0.17 | 1.49 | 0.16 | 0.03 |
| | Mand. | 27 | 1.39 | 0.17 | 1.37 | 0.16 | 0.03 |
| Females | Max. | 42 | 1.46 | 0.11 | 1.44 | 0.12 | 0.02 |
| | Mand. | 42 | 1.33 | 0.10 | 1.32 | 0.10 | 0.02 |

of males, although, as a rule, the greater the number of individual items in the series analyzed, the wider is the range of distribution. In this particular instance three additional facts increase the probability of correctness of the statistical finding here reported.

Firstly, although two different methods of statistical analysis were employed, the standard deviation figures obtained indicate a strong contrast between variability in the two sexes regardless of the method used.

Secondly, in each sex there is a strong similarity between the measures of variability for maxillary and mandibular teeth. Were this not the case, one would have some reason for questioning the statistical adequacy of the unprocessed data, and a confirmation of unequal degrees of variability would make it necessary to base the analysis on a larger number of observations.

Thirdly, the discovery of a sex difference in the developmental pattern of permanent first molars is not an unexpected finding in view of the sexual precocity of the female "rhesus" monkey. Several studies of human data have revealed similar positive correlations, especially in girls.[4, 25, 29] Again, had these teeth been found completely unaffected by the general rate of

physical maturation, one would have been led to question the sufficiency of the material under investigation.

B. *Variable duration of early phases of odontiasis.* To show how variability increases with advancing chronological age, Figures 1, 2, 3, and 4 have been extended to the left, or toward the neonatal period, to permit the inclusion of cumulative percentage graphs for the five classes of deciduous teeth which precede the emergence of the permanent first molars. The curves for the deciduous teeth have been drawn on the basis of cumulative percentage determinations for every hundredth of a year, starting just prior to birth.

TABLE 4. LENGTH OF FIRST PERIOD OF ODONTIASIS* AND OF INTERVAL OF REST PRECEDING EMERGENCE OF A PERMANENT FIRST MOLAR, FROM DATA ON 69 MACAQUES
All data in decimals of a year

| *Dental arch* | | *Emergence of deciduous dentition* | | | *Outwardly quiescent phase* | | |
|---|---|---|---|---|---|---|---|
| | | *Minimum* | *Average* | *Maximum* | *Minimum* | *Average* | *Maximum* |
| Males | Max. | 0.29 | 0.39 | 0.53 | 0.87 | 1.06 | 1.50 |
| | Mand. | 0.28 | 0.37 | 0.48 | 0.75 | 0.96 | 1.50 |
| Females | Max. | 0.28 | 0.38 | 0.52 | 0.81 | 1.02 | 1.18 |
| | Mand. | 0.25 | 0.35 | 0.42 | 0.79 | 0.93 | 1.13 |

* The period of emergence of the permanent teeth constitutes the second period of odontiasis.

In all four instances the nearly vertical slopes of the midranges of the deciduous incisor curves are in strong contrast with the lesser slopes of the curves depicting the emergence of deciduous second molars and permanent first molars. These contrasts, together with the existence of a relatively long outwardly quiescent period between the completion of the deciduous dentition and the advent of the first permanent tooth, naturally give rise to the question of whether the two major phases of dental growth and development are at all interrelated.

Table 4 summarizes certain information that can be obtained by analyzing the contents of Tables 1 and 2, but only surmised by inspecting Figures 1, 2, 3, and 4. According to this table the outwardly quiescent interval that separates the two major periods of odontiasis is always longer than the active period of emergence of the deciduous teeth which precedes it. It is evident, further, that dental growth processes in the mandible are, on an average, somewhat speedier than those in the maxilla, in both sexes.

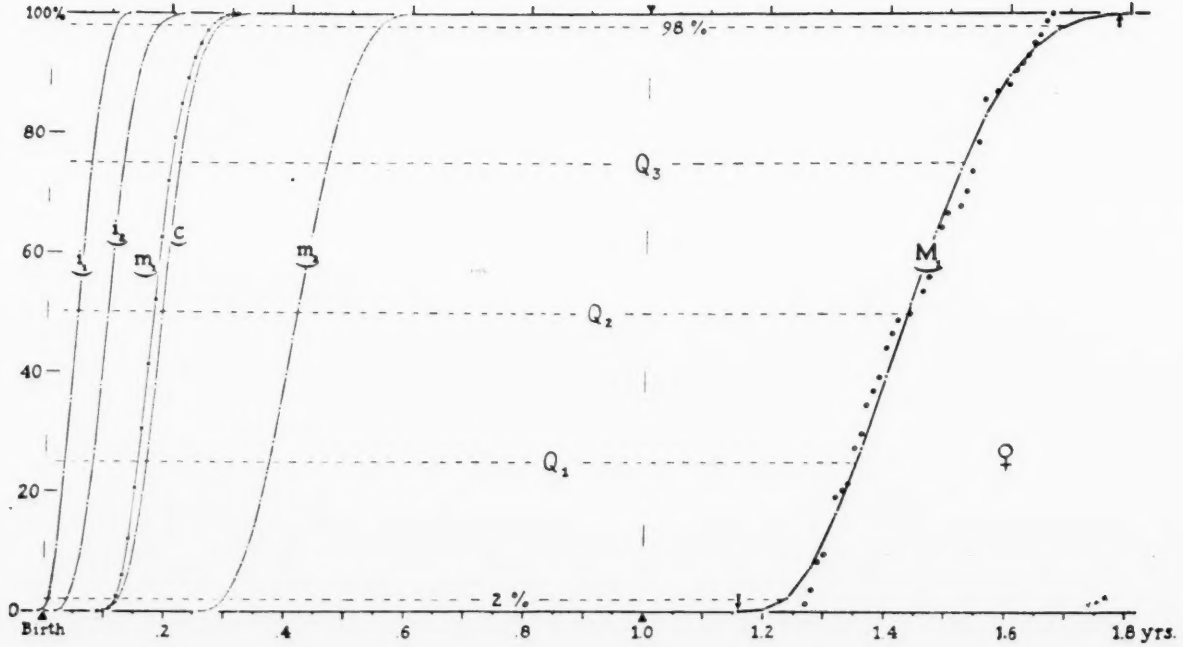


FIG. 3. Cumulative percentage ogives for the emergence of the permanent maxillary first molar ($\underline{M_1}$) and the five classes of deciduous maxillary teeth of female macaques (*Macaca mulatta*).

For explanation of details, see text under Figure 1.

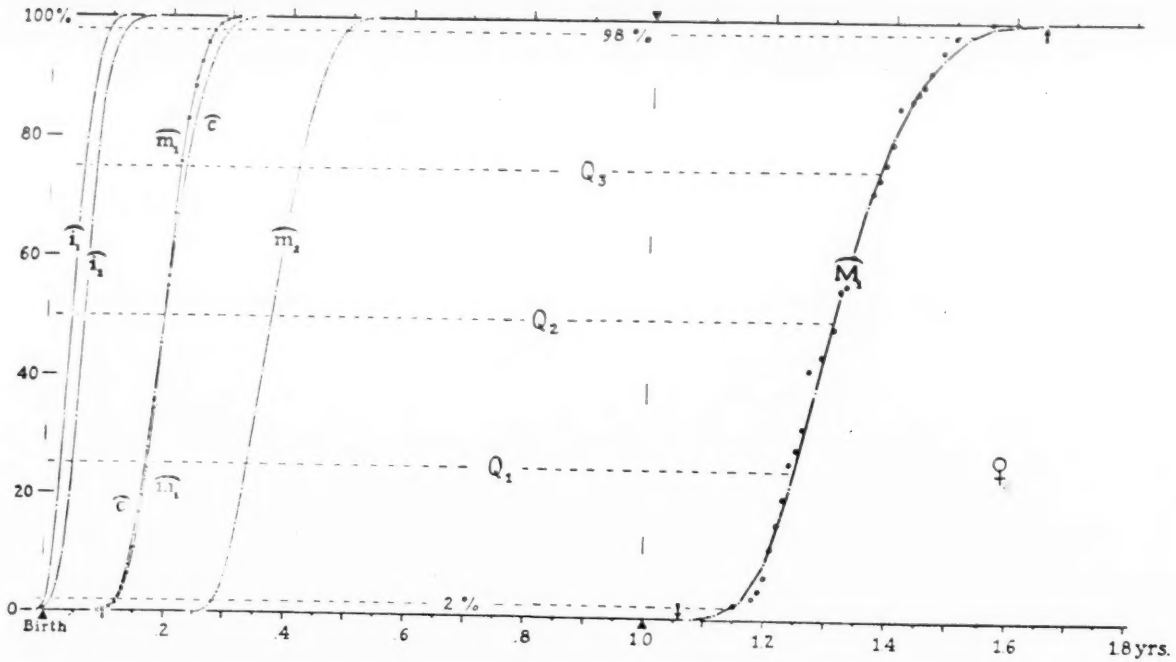


FIG. 4. Cumulative percentage ogives for the emergence of the permanent mandibular first molar ($\overline{M_1}$) and the five classes of deciduous mandibular teeth of female macaques (*Macaca mulatta*).

For explanation of details see text under Figure 1.

C. *Dental interrelations.* Mutual relationships between the growth and development of the many individual organs which collectively make up the dentition of a mammal such as the monkey—not to mention the human species—are still a virtual "*terra incognita*" to biology. There seems to be no literature at all on the interrelationships between the eruption of the deciduous and permanent teeth. If the reports by Nissen and Riesen[20] and Hurme and van Wagenen[13] are not considered, only a small number of articles by other writers can be cited as containing any documented information on the subject.

The data by Schultz[24] on monkeys are too limited to enable one to draw any definite conclusions, while those by Spiegel[28] merely *suggest* that early emergence of deciduous second molars is likely to be followed by earlier-than-average emergence of the permanent first molars.

After a study of 8 to 12 children Baldwin and Smith[1] concluded, in 1925, that "there was a tendency for children who began dentition early to finish early," and vice versa. The graphs which accompany the report by Camescasse[2] corroborate this finding, although they reveal considerable variability in individual instances. In one way or another the findings or tabulations of several other authors[6, 7, 9, 14, 15, 18, 26, 27] are in harmony with the concept of Fujita[8] who in 1947 stated explicitly that "there is an interrelationship between the eruption times of the teeth of an individual."

A recent article by Clements, Davies-Thomas, and Pickett,[5] however, contains an assertion to the effect that the times of eruption of certain teeth in man are "independent of each other," or "distributed at random." This generalization appears too sweeping in the form in which it is stated and may be due to the fact that the British study was based on cross-sectional material, analyzed by utilizing techniques which differ considerably from those employed in the present investigation, and that it attempted no review of the scanty literature available.

Having at their disposal many long-term records of serial observations, which are ideal for studies that deal with such complex phenomena as the development of the dentition, the present authors made an effort to find out—without too much sacrificing of detail—whether the analysis of a larger body of data would confirm the findings reported by them earlier.[18] It was desired particularly to throw some light on the possible interrelationships between the emergence of the deciduous teeth, individually and collectively, and the initiation of the process for the succedaneous dentition. The first permanent tooth cut by *Macaca mulatta* is a first molar, which is also ordinarily the first in man. This tooth is of special interest to dentists and odontologists, many of whom, rightly or wrongly, regard it as "the keystone" of a dental arch.

To prepare the ground for an assault upon the problem just discussed, the actual ages of emergence (see Tables 1 and 2) of all deciduous teeth and of all four permanent molars in the 26 males and 40 females (whose dental records were adequate for the purposes of this special study) were converted into so-called "Z" score readings. Following this average standard scores for all 12 tooth pairs in each of the 66 macaques were computed and arranged in rank order in two series, one for males and one for females. High negative values were placed at the beginning of each series and high positive values at the end.

The results were plotted on graph paper to construct the rather complex scatter diagrams reproduced here as Figures 5 and 6. Standard scores for a given animal were plotted along the same ordinate, the location of which was determined by the rank order of the average value. The plotting was done with care so that the diagrams would preserve, indirectly, the numerical values that determined the placement of the symbols.

The symbols used are explained under the word *Legend* at the bottom of each figure. With one exception, they have been selected with the possibility of a need for superimposition in mind, and a few examples of three signs combined can be found. Where superimposition would have resulted in too much confusion, the symbols have been arranged along the same horizontal line, or in a tight cluster, both to save space and to keep the identity of the vertical rows distinct. Also, to avoid excessive complexity of the graphs, no separate signs were used for the five morphological classes of deciduous teeth in each jaw; it was deemed sufficient only to make a distinction between maxillary and mandibular teeth. For the same reason right and left teeth were not differentiated either, but the average for a tooth and its antimere was used.

More data were available on female monkeys than on males. Therefore, in order to make mutual comparison of the two "scattergrams" easier, their horizontal dimensions were made approximately the same, while the vertical "Z" scales were kept constant, a procedure which necessitated wider spacing of the vertical rows of symbols for the males than for the females.

Figures 5 and 6 may be said to speak for themselves. They show, with great clarity, that eruption times are not completely independent of each other, in spite of fairly wide limits of variability. The symbols are not scattered evenly all over the diagrams, each of which shows them forming a wide zone which rises gradually from the lower left quadrant to the upper right quadrant. No statistical calculations are needed to prove that a given tempo of development does not affect the teeth as isolated units, but rather as integral parts of a larger unit—the dentition—which maintains its essen-

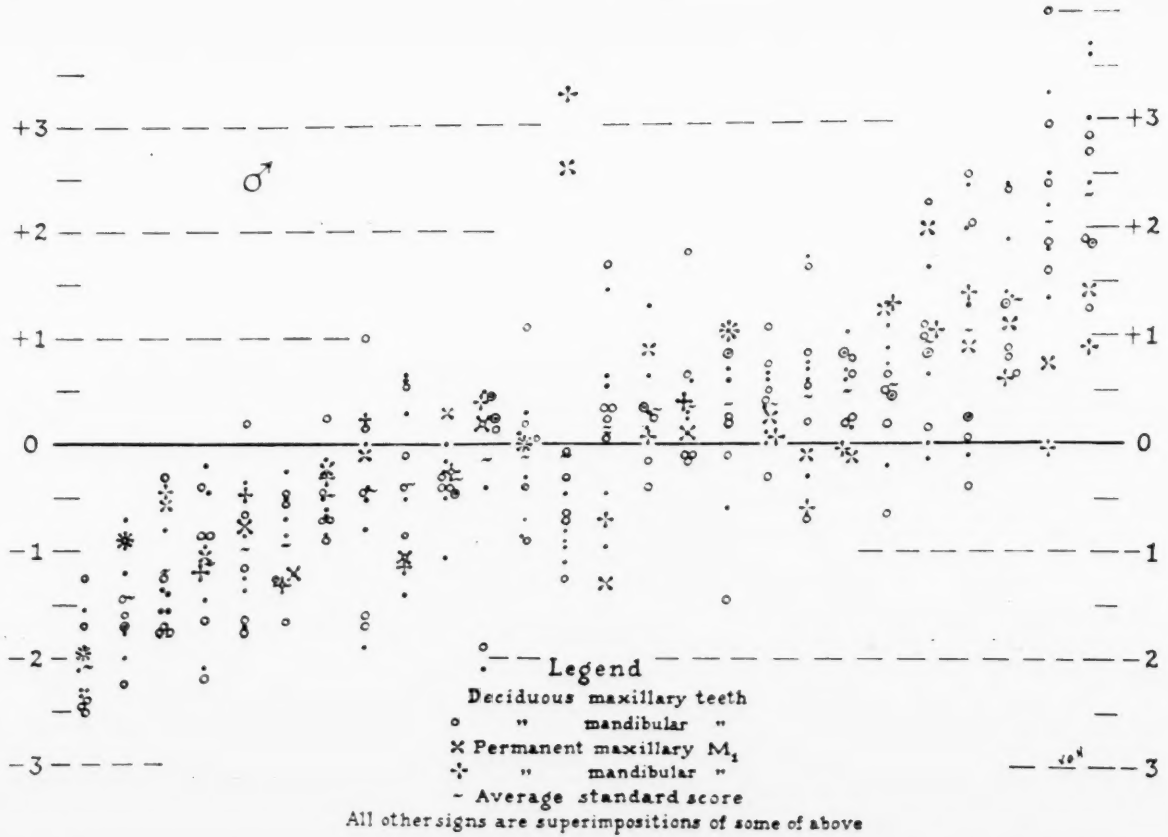


FIG. 5. Scatter diagram showing emergence ages of all deciduous teeth and of the permanent first molars in 20 normal male macaques. The data were converted into standard scores prior to plotting; the sequence of vertical rows of 12 signs is based on rank order of average values (~) for individual animals.

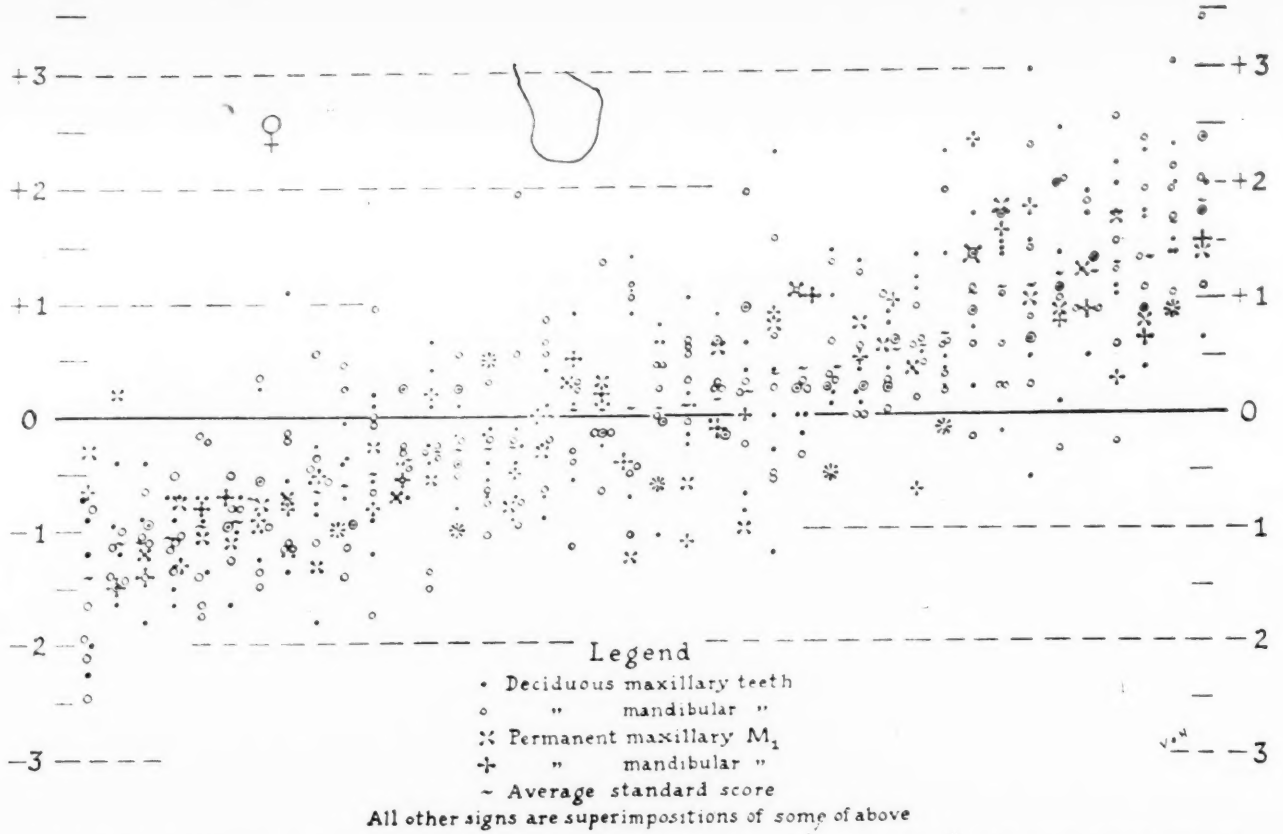


FIG. 6. Scatter diagram showing emergence ages of all deciduous teeth and of the permanent first molars in 40 normal female macaques. Plotting is in terms of standard score units above or below the zero line; sequence of vertical rows is determined by rank order of average values (~).

tial characteristics regardless of how rapidly or slowly the process of odontiasis takes place. If the first few deciduous teeth are early in their emergence, then the teeth that follow are also more or less early, and vice versa. A study of the contents of Tables 1 and 2 will confirm this generalization.

The permanent first molars do not behave in a manner that would indicate any perceptible degree of nondependence upon the same growth mechanism which brought about the emergence of the entire deciduous dentition that predates their much later arrival. (However, just as a reminder of Nature's ability occasionally to break a firm-appearing rule, the 13th item in Figure 5 shows exceedingly late eruption of the permanent teeth in a male whose entire deciduous set emerged relatively early.) The position of the permanent first molar symbols in Figures 5 and 6 varies as much and within the same limits as does the position of any of the other plotted signs, suggesting that in the vast majority of instances these teeth are part and parcel of the same picture, with distinctly limited autonomy.

The scatter diagrams also fail to reveal any clear segregation of the symbols which differentiate the maxillary and mandibular teeth. This is further evidence of the nonlocal character of the physiological mechanism which controls the growth and development of the dental organ complex.

It must be recognized, however, that Figures 5 and 6 do not completely solve the question of intercorrelations between various teeth in the same animal. Experimental studies might reveal that the separate units of a dentition have little or no *direct* influence on each other's development. The biometrician, moreover, will bear in mind the fact that although two variables are uncorrelated, it is possible to obtain a marked correlation if the data consist of mixed groups in which the means are correlated.

No one class of teeth was found definitely more prone to extreme deviations than another. The maxillary and mandibular first incisors and the mandibular second incisors of the deciduous set were, perhaps, slightly more erratic than the other teeth, in both sexes, while the permanent maxillary first molars displayed the greatest relative stability in each sex.

One additional fact may be noted after a study of Figures 5 and 6: chronological variability increases, on an average, which each degree of delay in dental maturation. The number of superimposed or closely clustered signs is more at the beginning of each series than at the end. This finding is in harmony with the previously discovered positive skewness of the frequency distributions.

It is apparent that in attempts to ascertain the velocity pattern of dental emergence in an individual case one's success will be the better, the more one knows about the emergence times of *different* classes of teeth in the same mouth. Dependence on information on a single tooth (or tooth pair)

could be misleading, since that one tooth may be the most nonrepresentative member of the entire dentition. The dispersal of signs in Figures 5 and 6 is additional proof of the validity of this contention.

The findings described so far prompted the authors to examine the phenomenon of multiple emergence against the background of general over-all rates of odontiasis. It did not seem reasonable to dismiss completely the question of the order of eruption simply because it had not been convenient to initiate the study of dental interrelations by concentrating on this special feature first.

Among the deciduous teeth those most frequently exhibiting departures from the usual order of emergence are the canines and first molars, as is well known. The mean eruption ages of these teeth are close together and the ranges of variation quite similar, so that irregularity in the sequence of their development should cause no surprise.

Figure 7 shows, by the heavy step-like line, the average "Z" scores for 25 normal male monkeys. Eight of these animals had either simultaneous (difference $< 0.01$ yr.) or nearly simultaneous (difference $= 0.01$ yr.) emergence of the deciduous first molar and canine in one to four quadrants of the mouth, the time of their emergence being indicated by the level of the quadrant symbols measured against the left hand scale.

Six out of the eight instances of some degree of multiple emergence are to be found in the first half of the series, which indicates that one may expect to find multiple emergence far more often among male macaques with accelerated dental maturation than among those with a slower rate of development.

Among males the canines usually precede the first molars in eruption, the ratio being about 3.5 to 1. By assuming slight delay of canine development and equally slight hastening of that of the adjoining first molar, the simultaneous emergence of these teeth is easily explained in monkeys with a relatively rapid over-all rate of odontiasis.

Figure 8 is a visualization of similar factual material on 39 females. The mean ages of emergence of the deciduous canines and first molars of the female are much closer together than those of the male. More frequent instances of multiple emergence of these teeth should therefore be expected in the female of the species, and Figure 8 shows that this is, indeed, true; only the end section of the series—the last 6 out of 39—is free of symbols indicating multiple emergence. Among females the first molars usually precede the canines in a ratio of about two to one.

Reversals of the usual order of emergence of the deciduous canines and first molars present an interesting distribution among the males. The first molars do not pierce the gingiva ahead of the canines among any of the

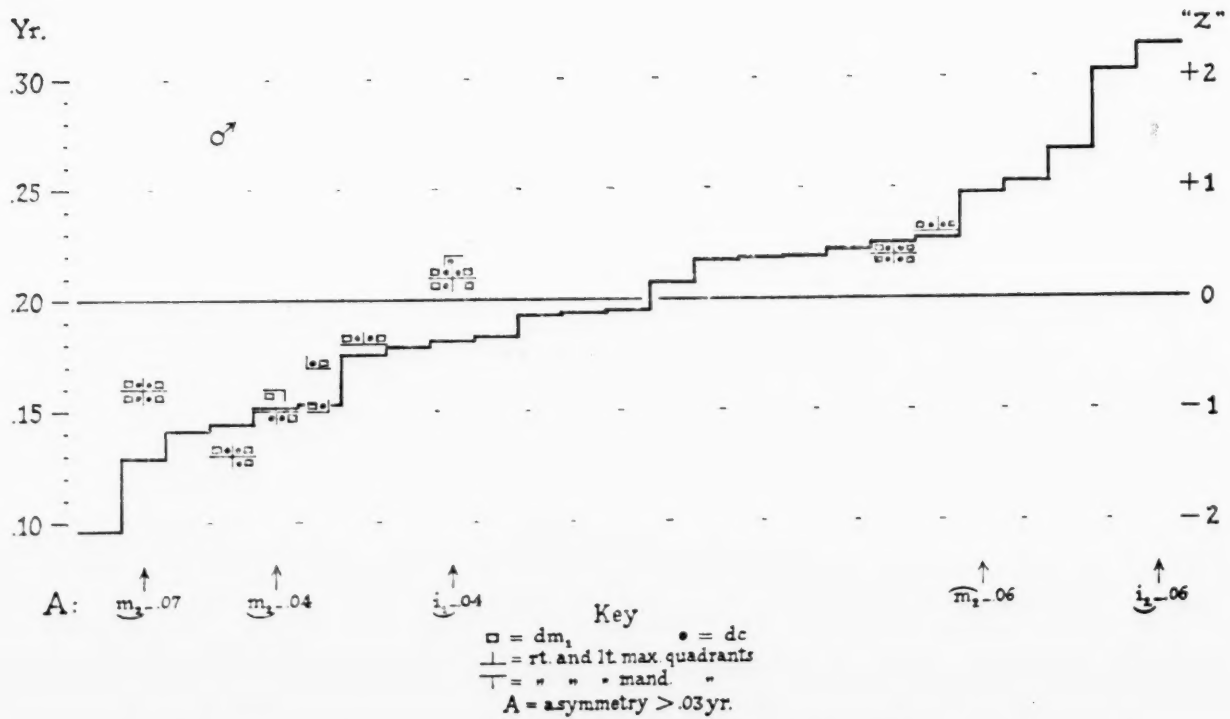


FIG. 7. Distribution of instances of multiple emergence of deciduous canines and first molars in 25 normal male macaques. Average "Z" scores are shown by the heavy step-like line. Instances of marked chronological asymmetry (> 0.03 yr.) in the emergence of various deciduous teeth are noted along the bottom line. (See p. 557)

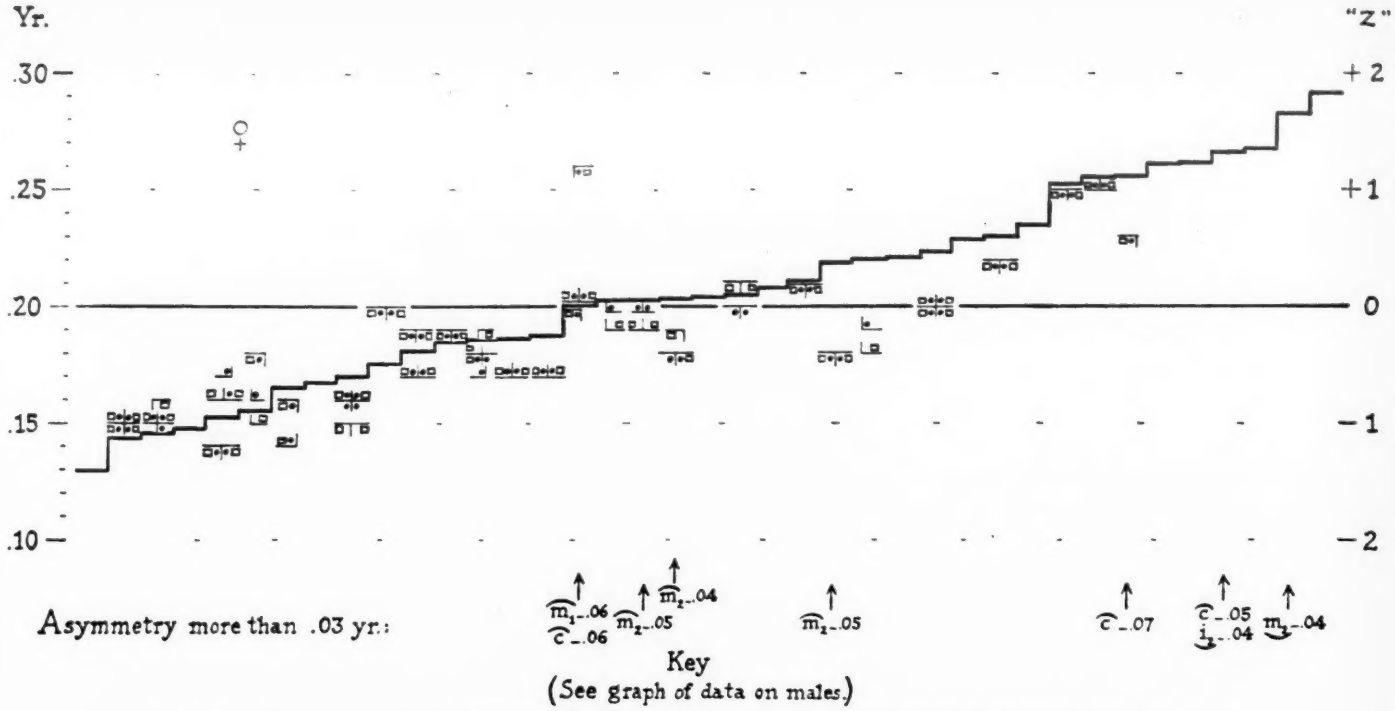


FIG. 8. Distribution of instances of multiple emergence of deciduous canines and first molars in 39 normal female macaques. For explanation of details see text under Figure 7.

animals at the beginning of the series; only among those with slower rates of dental development does this reversal occasionally take place. Its occurrence is suggestive of a tendency of some of the more slowly maturing males to adopt the normal female pattern of first molar precedence.

At the bottom of Figures 7 and 8 are some notes on the occurrence of more marked asymmetries in the emergence of deciduous teeth. It will be noted that, apart from asymmetries involving the deciduous second molar, those involving other units of the primary dentition seem to occur least often among the animals with speediest dental development.

D. *Physical-dental interrelations.* On most of the animals whose dental records had furnished the subject matter of the present study there were other growth data that had been collected in a uniform, systematic manner. The availability of these additional data provided the writers with an opportunity to test the correctness of some of the conclusions already reached and, coincidentally, to demonstrate the astonishing scope of the relationships that exist between the teeth and physique of a young primate.

(a) Growth rates and ageing. The tieup between general metabolic activity and the growth and development of the masticatory organs has been revealed already by the analyses showing several distinct contrasts between the dental data on male and female monkeys. Perhaps the most striking of these is the sex difference in the variability of emergence of the permanent first molars, which suggests the domination of local dental phenomena by the general physiological activities of the entire body.

Another piece of evidence of the interrelations just mentioned is the increase in the variability of tooth emergence which is seen when the latter is related to ageing. As the metabolic processes of the body gradually slow down, the mechanism that initiates the process of tooth emergence also slows down, with the result that positive standard score deviations for various teeth become greater and greater (or the negative deviations smaller and smaller), and the chances for multiple emergence fewer and fewer.

(b) Menarche. The age at menarche had been recorded for 39 of the 42 normal female macaques included in the present study. This made it possible to study the relationship, if any, between an obvious physical sign of sexual maturation and the over-all rate of tooth emergence, and to determine whether the latter phenomenon is directly correlated with the functioning of the sex organs. It could not be assumed, on the basis of statistical sex differences in the eruption of teeth, that the teeth were in any extended sense of the term secondary sex characteristics in the "rhesus" monkey, for it was equally easy to assume that their development simply reflected the development of the body generally, quite apart from the activity of the

ovaries. Or, to phrase the above thought differently, it was possible that dental maturation was not correlated with sexual maturation, but merely *associated* with it, as a rule, and only *indirectly* influenced by the hormones that have something to do with sex.

The beginning of menstrual function in the macaque shows no chronological constancy in different females. The range of variation is quite wide, extending from 1.43 to 2.67 years, with an average at 2.00 years. For this reason, although a cursory examination of the data revealed no association, positive or negative, between rate of odontiasis and age at menarche, the coefficient of correlation for the two variables was computed. The exceedingly low value of $r$ that was obtained—$+0.02$—certainly strengthens the argument that the two variables are only casually interrelated, as suggested by Shuttleworth.[20]

(c) Length of gestation. Long before either physicians or dentists had paid any serious attention to the connection between the time of emergence of the first deciduous teeth and the length of the gestation period, cattle growers had noted that the two events were not altogether isolated from each other. Youatt, in his remarkable treatise on the management of cattle, published in 1834, wrote as follows: "The mouth of the newly-born calf presents an uncertain appearance, depending on the mother having exceeded or fallen short of the average period of utero-gestation," but without attempting to document this assertion.[24] He is, perhaps, the first observer to make a written record of the above mentioned relationship, although the possibility of some other author predating him has not been ruled out.

Even much more recent works that deal with factors controlling the time of eruption of the primary teeth generally do not include any reference to the length of the prenatal period of life. The one exception to the above rule is the teeth of premature children; a few authors, like Ledé,[17] Nëu,[19] and Campini and Urquijo[2] have recorded evidence of their retarded emergence, if measured in terms of postnatal age.

Hurme and van Wagenen[13] probably have done the most so far to substantiate Youatt's observation. Although the proof offered by them, based on an analysis of data on the monkey, is fairly conclusive, it appears possible to improve on it further by re-examining the problem in another manner, utilizing the standard score technique employed earlier in other analyses mentioned in this report.

The length of fetal life was known for 23 male macaques out of the 27 included in the present study, and for 31 females out of 42. After arranging the average standard score values computed for the 12 pairs of emerging teeth in each animal in rank order—as had been done previously in prepar-

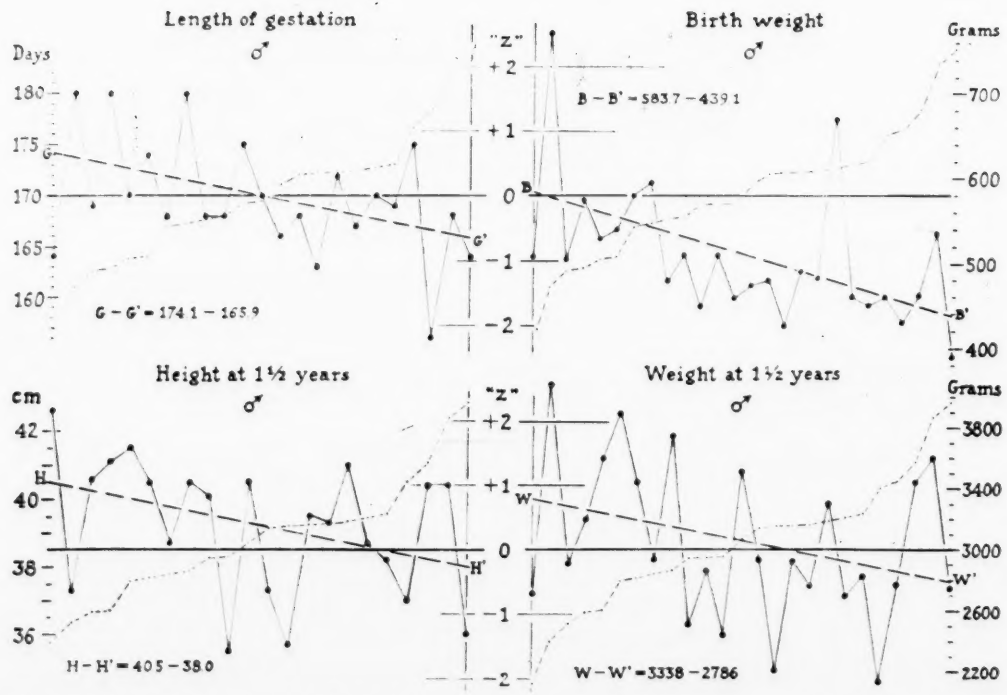


FIG. 9. Relationship between rate of odontiasis, length of gestation, and three measurements of physical size in 22 to 26 normal male macaques.

For explanation of details, see TEXT on p. 561

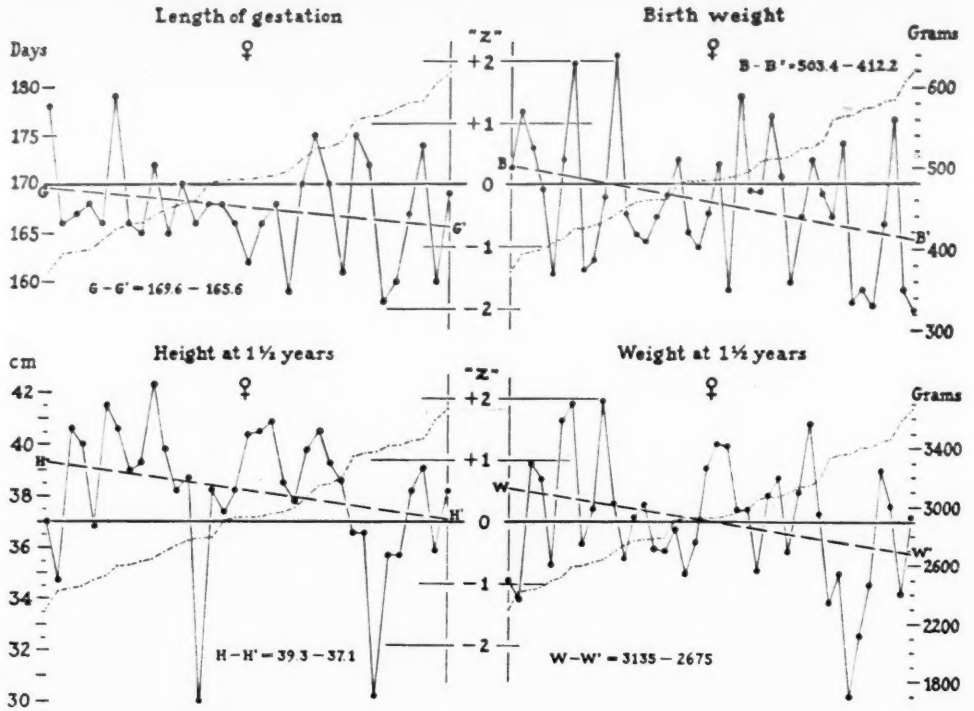


FIG. 10. Relationship between rate of odontiasis, length of gestation, and three measurements of physical size in 31 to 40 normal female macaques.

For explanation of details, see text on p. 561.

ing Figures 5 and 6—corresponding gestation ages were placed in the sequence determined by the average standard scores. The trends shown by the gestation ages—after arrangement in the order just mentioned—were then analyzed statistically.

Again, in order to conserve space and to present the findings in a readily assimilated form, the data were incorporated in a graph, this being done with sufficient care to permit extraction of original numerical values from the graphs if desired later by a reader. The material pertaining to the analysis of dental data versus gestation data is presented in the left upper quadrants of Figures 9 and 10. In each of these figures the fine broken line ascending from left to right represents the average standard score (or "Z") values, the heavy black dots (connected by solid lines), the length of gestation periods, and the heavy broken line slanting down from left to right, the mathematical summarization of the trend indicated by the heavy black dots, considered collectively. The exact numerical values corresponding to the ends (G — G') of this trend—or regression—line are also given in each diagram.

An examination of the graphs leads one to conclude that as the length of the gestation period decreases, on an average, the speed of odontiasis decreases.

In spite of much individual variation, the trends shown in Figures 9 and 10 are clear, the data on males displaying the relationship most convincingly. In this connection one may observe that the repeatedly encountered greater variability of the male macaque is revealed also by the statistics on the length of gestation when these are computed by utilizing more extensive raw data.*

A truly adequate interpretation of the interesting relationship between the length of gestation and the over-all rate of odontiasis is beyond the ability of the writers. What can be said at this point is that when the intrauterine sojourn of a macaque exceeds the average of 168.8 days, it is more likely to arrive into the outer world with a greater potential of growth vigor than is the animal whose prenatal development falls short of the average. Earlier and speedier odontiasis is partial proof of the plausibility of this proposition, which, in itself, is neither new nor revolutionary. It can be said, however, that the aforementioned proposition has never before been put to a test by subjecting dental growth data to a rigorous analysis.

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* The means and standard deviations, estimated from data on 42 normal males and 46 normal females, are 168.6 ± 5.6 and 169.0 ± 5.2 days, respectively. Data on four twins were not included in these two "normal" groups. The figures obtained differ slightly from those published earlier.[3]

To rule out the slightest possibility that the delays or prematurities of permanent first molar emergence discussed in this paper would be cancelled off if the ages under consideration were expressed in terms of postconception intervals of time, the data on this class of teeth were placed under scrutiny once more. It was known that the ranges of variability for the permanent first molars were of much greater magnitude than the ranges of variability for the gestation ages of animals born "at term," even if it is assumed that a few born a little before or a little past term are included. Therefore, if the mathematical summation of the ages of emergence of the permanent first molars should yield regression lines that are no steeper than the lines (G — G′) summarizing the data on periods of gestation (in Figs. 9 and 10), it would be self-evident that one should not infer that a decrease in the length of gestation is generally followed by a *real* delay in the time of emergence of the first permanent tooth. Furthermore, should the lines obtained slope down from left to right, like the lines G — G′ in Figures 9 and 10, it would be obvious that the original inference is diametrically opposite to the truth and wholly untenable.

The data on the permanent maxillary first molars of 23 male macaques with gestation periods of known length were selected as a suitable test sample for the analysis mentioned in the preceding paragraph.

The summation of data on their emergence yields a regression line *rising* from 471.6 days (1.292 yrs.) to 589.1 days (1.614 yrs.). The difference of 117.5 days is indisputably greater than that obtained for the ends of the regression line (G — G′) which summarizes the data on the periods of gestation in the same group of animals. (See Figure 9). In the latter case the difference is only 8.2 days, which is much too small to allow an explanation on the basis of chronological variations in length of gestation.

An inspection of the placement of permanent maxillary first molar symbols (X) in Figure 5 is perhaps even more convincing, and the test can be applied with equal success to the data on mandibular first molars, in the same figure, or to the material on female monkeys, shown in Figure 6.

(d) Birth weight and physical size later. One would expect an infant with a longer-than-average prenatal existence to be heavier than average at birth, as a rule. In view of this it is not surprising to find a positive correlation between birth weight and speed of odontiasis in the material at the disposal of the authors.

The heavier the baby, the more likely is it to have early and rapid emergence of its teeth, as is evident from an examination of Figures 9 and 10, wherein the data are shown in the upper right hand quadrants. Although this generalization applies to both males and females, it is particularly true

of the males. The female curve of birth weights is characterized by much more kurtosis than the curve for males.

What is rather surprising is the persistence of this association at the age of one and a half years, long after the emergence of all deciduous teeth. Taking the males and females as a group, the heavier animals have earlier eruption of their teeth than the lighter ones. The relationship extends even to the height of the monkeys at 1½ years, although it is not quite as clear-cut as in comparing the rate of dental maturation with weight. The data on which the above inferences were based are shown in the two lower graphs in Figures 9 and 10.

A review of literature discloses the existence of very little confirmatory evidence for the findings just discussed. Baldwin and Smith[1] found the later-born children heavier and taller than those born earlier; they also found "a strong tendency for the children to hold until maturity the rank in height that they held at birth." (Presumably the same could be said about rank in weight.) As regards birth weight and teething, the only report that has come to the attention of the writers is that by Yamada,[21] whose data fully substantiate the findings obtained as a result of the present investigation.

## SUMMARY

This paper describes analyses of certain long-term records on the growth and development of 69 normal macaques born and reared in captivity. Particular attention has been paid to permanent first molar emergence and to other growth changes observable in the monkey prior to 2 years of age. Literature dealing with interrelationships of general physical and special dental development has been reviewed.

The findings and conclusions can be summarized under five main headings:

1. *Variability.* The emergence of teeth is characterized by considerable variability, which increases with age, if measured in conventional units of time. The hypothetical extremes of permanent first molar emergence in the macaque are approximately 12 months apart for the teeth of males and 7 months apart for the teeth of females.

Variability does not always affect the two halves of a dentition equally, with the result that chronological asymmetry may appear in the eruption of a tooth and its antimere. Generally speaking, as variability increases with age, so do temporal asymmetries in the emergence of right and left teeth.

Postnatal variability in the ages of emergence is partially accounted for by variations in the length of gestation. The longer the period of intra-

uterine development, the sooner after birth do the teeth appear, as a rule. Nevertheless, the ranges of variation in the emergence of permanent first molars are so wide as to make it obvious that the inequalities must be explained in terms of physiological differences rather than in terms of discrepancies in the chronology of histodifferentiation.

The findings indicate a close association between developmental rates and tendencies to deviations from the biometrician's average. Rapid rates of odontiasis are characterized by lesser degrees of chronological variability than slow rates of dental maturation.

2. *Relative constancy of emergence patterns.* Variability in the times of emergence of individual teeth does not result in much variation in the transitional anatomical patterns of developing dentitions. Although the eruption of one tooth is interlinked with that of another, this linkage is not so rigid as to make observations on the sequence of emergence of various teeth the best method for demonstrating the high degrees of constancy in the developmental patterns of different animals.

The most common order of emergence is given by the mean values obtained for each class of teeth. Only teeth with similar mean values produce frequent deviations from the prevailing pattern. The first molars initiate odontiasis of the permanent series, the mandibular molars usually preceding the maxillary ones.

Observations on variations in the pattern of emergence may furnish evidence of the general rate of development and of sexual maturation in the monkey. This idea is substantiated by the finding that multiple emergence of certain deciduous teeth seldom takes place in a slowly maturing animal, and that a high proportion of males with a slow rate of dental development reveal a transitory anatomical pattern which is generally characteristic of females.

3. *Relative constancy of developmental rates.* The rate of operation of the physiological mechanism that controls the initiation of the process of emergence of various teeth appears to be remarkably constant in the monkey during its first two years of life.

The rôle of velocities of growth and development is all-important in the establishment of whatever dental interrelations can be observed. If these velocities fluctuated very much from one age period to another, the biometrist would not find much statistical orderliness in the processes of tooth eruption.

The present analyses suggest that the rates of odontiasis are causally related, in some manner, either to the length of gestation or to something else which determines the duration of the latter. In either case it is apparent

that the basic "rhythm" of tooth emergence is determined prior to birth, and that it is less alterable than the behavior of an individual dental organ. Infants with long gestation periods average earlier and speedier development of their dentition than those with a relatively short prenatal span of existence.

The high degree of constancy of the basic rate of dental development in an individual is revealed most clearly by the finding that it is not altered much by the relatively long outwardly inactive interval of time which separates the two main periods of odontiasis. The time of emergence of the permanent first molars can be predicted with moderate success if the times of emergence of the deciduous teeth are known.

4. *Physiological age.* All findings on physical-dental intercorrelations emphasize the idea that the growth and development of the dental organ complex is dependent on the growth and development of the entire body. It may be said, nevertheless, that although the above idea is not new, rather limited use has been made of teeth as indicators of physiological age, apart from their value as indicators of certain pathological disturbances.

The dentition certainly appears to offer an investigator most valuable clues for appraising the over-all rate of maturation of an individual. This is demonstrated most strikingly by the different degrees of variability in the emergence of the permanent first molars; the earlier maturing female exhibiting markedly less variability than the later maturing, less predictable, male monkey.

5. *Practical implications.* Eruption of teeth serves as an excellent indicator of both physiological and chronological age. It is regrettable that to make use of this indicator requires the keeping of detailed long-term records on a large number of separate dental units.

The results of the present investigation make it appear possible to draw some reasonably reliable conclusions from observations on a limited number of teeth, if suitable statistical standards of reference are available. The authors believe that they have furnished such standards for the normal *Macaca mulatta*. For the sake of convenience in any biological appraisal males and females should be treated as if they represented two different species.

It seems safe to hypothesize that animals which mature at different rates are unequally susceptible to similar environmental stimuli or agents. Attention to rates of dental maturation should enable the experimental biologist to design more critical laboratory studies. The odontologist is in a strategic position for supplying information that is necessary for formulating a theoretical basis for the differentiation of phenotypes and genotypes.

The meaning of minor local variations—affecting only one or a few teeth—can be understood better if the few static, genetically favored anatomical patterns for the species are known. Slight environmentally induced disturbances of growth processes in a semi-autonomous part such as a tooth probably can, under certain circumstances, lead to profound modifications of the anatomical configuration of the dentition and even result in permanently abnormal relationships that are not necessarily of genetic origin.

ACKNOWLEDGMENTS

The location and translation of the Japanese references presented a number of difficulties. That they were solved successfully is due to the help received from several colleagues, including Dr. S. Takahashi of the Orthodontic Department of Tokyo Medical and Dental University, Dr. M. Horiuchi, Clinical Fellow at the Forsyth Dental Infirmary for Children, and Dr. S. Ochiai, Intern during 1954-55 at the latter institution. Miss Dorothy J. Butzko, of the Department of Obstetrics and Gynecology, Yale University School of Medicine, rendered invaluable secretarial assistance many times in the course of the investigation. The contributions of these persons, and of several others not specifically named here, are gratefully acknowledged by the authors.

The speedy completion of the study is largely due to the wholehearted administrative support and encouragement furnished by Dr. H. M. Marjerison, the late Director of the Forsyth Dental Infirmary, to whom the authors are indebted for making this co-operative project possible.

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## EVENTS

### NU SIGMA NU LECTURE

December 7, 1955

RECENT ADVANCES IN THE STUDY OF STRESS. By Hans Selye, Professor and Director of the Institute of Experimental Medicine and Surgery, University of Montreal.

Stress is a specific syndrome induced by non-specific changes in the organism or a part of it. Among the manifestations of stress are gastric ulceration, thymic involution, lymphatic involution, eosinopenia, and an atrophy of the adrenal cortex. A stressing agent acts upon a target tissue with a direct effect of primary damage and local tissue response. These immediate effects are modified by an indirect or total body response which can act in a "prophlogistic" or "antiphlogistic" manner to augment, or depress, the initial form of the reaction to injury.

A series of experiments using rats in which the animals were injured in various ways followed by administration of appropriate doses of desoxycorticosterone or cortisone showed that cortisone depressed inflammatory responses and that desoxycorticosterone intensified them as compared to untreated controls. It was concluded that in general mineralocorticoids are "prophlogistic" and glucosteroids are "antiphlogistic." However, the apparently curative effects of the anti-inflammatory hormones are not desirable in all cases. Normal rats treated with ACTH, which elicits a predominantly anti-inflammatory adrenal cortical hormone response, were found to develop abscesses caused by ordinarily non-invasive bacteria illustrating that resistance was lowered by the failure of the inflammatory reaction to develop.

The effects of stress in perforating gastric ulcers were shown using the "granuloma pouch" technique in rats. The protection against peptic digestion and perforation afforded by the granulomatous response in gastric ulcers was overcome when psychic stress, ACTH, or cortisone was administered.

It was concluded that Pasteur's idea of the pathogenesis of a disease might well be modified to include the effect of the total body response to the specific etiologic agent. This concept may be applicable in the exploration of many of the diseases such as the allergies and the collagen diseases which are poorly understood at present.

R. S. N.

### YALE MEDICAL SOCIETY MEETING

January 9, 1956

THE INFLUENCE OF DIET ON THE SERUM LIPIDS IN DIABETIC RETINOPATHY. By W. F. Van Eck, Departments of Medicine and Public Health.

After one year on an experimental diet with fat restricted to 20 grams per day, four patients with diabetic retinopathy showed total remission of retinal exudates. The dietary carbohydrate was increased to keep the total caloric intake equal to that of the pre-experimental diet; this did not cause an increase in the amount of insulin required by the patients.

J. P. G.

## NEUROLOGICAL STUDY UNIT

January 10, 1956

EXPERIMENTAL MALFORMATIONS OF THE BRAIN. By Samuel P. Hicks, New England Deaconess Hospital, Boston.

The attempt to bring about reproducible congenital anomalies has led to the use of over seventy different kinds of injurious agents including X-radiation, anoxia, drugs, hormones, and trauma. Of these, X-radiation proved to be the most selective and formed the main basis for the present work. The procedure involved fairly accurate notation of the time of conception and radiation of the pregnant rat with about 200 r. Removal of one or two fetuses by Caesarian section at predetermined intervals allowed observation of the immediate foetal radiation injury. Its effect on development and growth was watched in a serial manner on litter mates. By varying the time between conception and radiation various cranial anomalies were produced. Thus, radiation on the 9th, 10th, 11th, and 12th days produced, respectively, anencephaly, encephalocele, narrow aqueduct and hydrocephalus, and parencephaly. Radiation during the 13th to 21st days resulted in microcephaly.

In the rat central nervous system all cells seem to be injured by radiation, but the differentiated cell does not proliferate while the least differentiated does. It was also noted that the mature neural cell is very sensitive to oxygen and glucose blood levels, while the neuroectoderm has a much lower sensitivity.

Malformations are determined by the developmental stage of the organism at the time of injury, by the nature of the injury, and the repair process stimulated.

P. B., JR.

## NU SIGMA NU LECTURE

January 18, 1956

RENAL TUBULAR EXCRETION. By John V. Taggart, Associate Professor of Medicine, College of Physicians and Surgeons, Columbia University.

The secretion of para-amino-hippurate (PAH), diodrast, and phenolsulphonephthalein (PSP), from blood to tubular lumen against a wide concentration gradient may profitably be studied as a model of that type of renal tubular activity which requires energy expenditure. Isolated flounder tubule will concentrate PSP 100 to 200 times when placed in a suitable medium; however, the addition of $2 \times 10^{-4}$ molar 2, 4, dinitrophenol (DNP) completely inhibits this concentrating power. Since DNP does not interfere with cellular respiration but uncouples phosphorylation, adenosinetriphosphate (ATP) may well be the energy source for this specific transport mechanism. *In vivo* observations on dogs with blood levels of PAH sufficient to exceed the normal Tm provide confirmation of this hypothesis; plasma concentrations of DNP similar to those used *in vitro* provide comparable inhibition.

Slices of cortex from rabbit kidney can concentrate PAH. Interestingly, while glucose and the phosphorylated intermediates of anaerobic glycolysis have little effect on this concentrating power, lactate, pyruvate, and especially acetate enhance it. Further studies show that dicarboxylic acids of the citric acid cycle, fatty acids of $C_6$-$C_{10}$ chain length, and l-glutamate and l-alanine markedly inhibit PAH transport. The *in vitro* synthesis of hippurate from benzoyl-Co-A and glycine and the isolation from kidney of the necessary enzyme, glycine-n-acylase, provide a clue to the explanation of these findings. Utilizing the fatty acids this enzyme can catalyze the formation of aliphatic acylglycines which, in turn, may compete with PAH for an unknown cellular transport mechanism, X. Apparently, acetate and its precursors form acetyl glycine which does not compete with PAH for X; a less competitive substance therefore forms and PAH transport increases. Confirming this idea, acetate stimulates PAH transport only in those species which have the glycine-n-acylase enzyme.

The general scheme of PAH transport may be represented as $PAH + X \rightleftharpoons X \cdot PAH \rightleftharpoons PAH + X$ where X again represents an unknown cellular intermediate. Work may be needed to form the intermediate complex or to rejuvenate the cellular component. Blockage of the available chemical linkage sites reveals that only interference with the $—COO^-$ radical influences the transport; hence this must be the linkage site. Studies with $—C{=}O^{18}$ show that no significant $O^{18}$ is lost in transport; therefore, the bond involved must leave the carboxyl radical intact after transport. Investigation of the various possibilities indicates that the most likely type of bond is purely ionic. This result is especially interesting in view of recent advances in understanding of cation exchange in the renal tubules. Perhaps an analogous anionic mechanism is involved.

R. I. B.

## ALPHA KAPPA KAPPA ANNUAL LECTURE

January 19, 1956

Heart Disease and Ways of Life. By Paul Dudley White, Harvard Medical School.

The commonest types of heart disease in the United States today are coronary, congenital, rheumatic, cor pulmonale, thyrotoxic, subacute bacterial endocarditis, cardiovascular syphilis, and tuberculous pericarditis. Of these the former is most prevalent and has manifested in the past decade a strikingly increasing incidence. Coronary disease, as regards incidence, prognosis, and possibly etiology, is influenced by many factors; these can be divided into those over which man has, and does not have, control of some kind.

The degree to which such factors as race, sex, and age play a rôle is largely speculative; that they are in fact somehow involved in the natural history of coronary disease appears highly probable. Heredity, insofar as it involves the transmission of physical characteristics such as coronary artery wall abnormalities, coronary collateral circulation, body build, and "chemi-

cal constitution," would also seem to be implicated. Certain personality types, e.g. the aggressive individual, are described as more prone to develop coronary disease; whether personality is in part an inherited characteristic is open to debate. The above factors are among those over which man has little or no control.

Still other factors must be considered as possibly related to the pathogenesis of coronary disease. These are such that man has, or could have, varying degrees of control over them. The "stresses and strains of our modern pace," both emotional and physical, have been described as contributing to the rising incidence of this disease. Personal habits such as excessive use of alcohol and tobacco, amount of physical exercise and sexual over-indulgence have been implicated. Local customs, e.g. religious fasting, and the afternoon siesta, are thought to favor decreased incidence. The high fat, high cholesterol diet has been a most commonly indicated factor. Whether it is the fat content per se, or the resulting combination of protein and carbohydrate, is a question upon which there exists considerable disagreement.

J. C. C.

ARTHRITIS STUDY UNIT

January 19, 1956

PRIMARY AND SECONDARY GOUT. By Alexander B. Gutman, Mt. Sinai Hospital, New York City.

Primary gout is an inborn error of metabolism. Secondary gout, a complication of prolonged hematological diseases such as polycythemia vera and chronic leukemia, follows the same clinical course. Both primary and secondary gout are characterized by hyperuricemia. If patients with primary gout are fed $N^{15}$ glycine, a precursor of uric acid, the labelled uric acid excretion curve in some cases is identical with that of normal subjects, i.e., a normal peak occurs in two to three days. In others, though the peak appears at the same time it is four to five times greater, indicating increased uric acid synthesis. Into which group a patient will fall cannot be predicted from the clinical course, but the latter group excretes excessive amounts of uric acid in the urine. In secondary gout the peak is slowly reached in about two weeks and is usually somewhat higher and broader than normal. Hyperuricemia in secondary gout is possibly related to excessive uric acid formation associated with increased turnover of nucleoproteins in these chronic hemopoietic diseases. Thus there are at least two and probably more metabolic pathways of uric acid formation.

Another possible explanation for hyperuricemia in gout is that too little uric acid is excreted. Of the uric acid in the glomerular filtrate, 90-95% is reabsorbed in the tubules. Probenecid inhibits this reabsorption by its action on the proximal convoluted tubules. One half to two grams of probenecid per day will result in a 30-50% increase in uric acid excretion and blood levels fall to half their original values. The effect of a single dose lasts about 24 hours and the drug can be given safely for months or years.

Of 43 patients with tophaceous gout treated with probenecid for eight months to four years and kept on a restricted diet, 94% experienced improvement in joint stiffness and pain; tophi were reduced in size in more than 50% and in two patients tophaceous sinuses healed. With probenecid even old tophi can be mobilized.

Salicylates are potent uricosuric agents and this action can be potentiated by bicarbonate. However, small amounts of salicylate have the reverse effect. This paradoxical action is observed with other uricosuric agents. Perhaps small doses increase tubular reabsorption while large doses are toxic. Another explanation might be that a small amount of uric acid is excreted by the tubules and that a small dose only inhibits the secretory phase while a large dose inhibits the reabsorption as well.

Among other uricosuric agents some of the derivatives of phenylbutazone have proven at least as potent as probenecid. In particular, the phenyl-thioethyl derivative shows good uricosuric action, only slight sodium retention and fair anti-inflammatory properties. However, there has not been enough clinical use of this drug as yet for a proper evaluation.

With regard to acute attacks of gout it is conjectured that they are not due to uric acid per se but to another purine not yet discovered. Chromatographic analysis of urine has revealed several new methyl purine metabolites. Of these, 8-hydroxy-7-methylguanine is excreted in two to four times the normal quantity in patients with acute gouty arthritis and polycythemia vera. However, no causal relationship has been demonstrated thus far.

L. Z. B.

## PHARMACOLOGY SEMINAR

January 24, 1956

BIOCHEMICAL APPROACHES TO THE STUDY OF BRAIN FUNCTION. By Bernard B. Brodie, Chief, Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda, Maryland.

In investigating the potentiating effects of reserpine and chlorpromazine on barbiturates, the structural similarity between 5-hydroxytryptamine (serotonin), lysergic acid diethylamide (LSD), and reserpine was noted and all were seen to contain an indole nucleus. Serotonin, a naturally occurring vasoconstrictor, was given to mice—whose vasomotor system is not sensitive to the drug—with resultant sedation similar to that produced by reserpine. Mice pretreated with serotonin had a greatly potentiated reaction to hexobarbital. If mice were pretreated with LSD, the potentiating effect of serotonin was considerably reduced. In dogs and rabbits examination of the urine for the excretory product of serotonin, 5-hydroxy indole acetic acid, revealed a high level with a return to normal in eight hours following injection of reserpine.

A spectrophotofluorometric method for serotonin analysis was developed. With this technique the serotonin level was found to be high in the primitive parts of the brain, particularly the hypothalamus and brain stem, but absent in the cerebellum. A considerable reduction in serotonin content of

the small gut, platelets, and brain was found following the injection of reserpine. Barbiturates and isoreserpine, a stereoisomer, were without such effect.

After high doses of reserpine, brain levels of reserpine and serotonin in dogs were determined, with the former disappearing in four hours and the latter remaining low for as long as three to five days. It is postulated that reserpine causes the release of serotonin from cells followed by a rapid enzymatic destruction and excretion. Thus, there is a slow return to normal intracellular levels.

A working hypothesis of the mode of action of reserpine in psychiatric disorders, based on the above considerations, was presented.

P. B., JR.

## ZOOLOGICAL JOURNAL CLUB

February 8, 1956

THE OMMATIDIUM AND COMPOUND EYE PHYSIOLOGY. By Talbot H. Waterman, Associate Professor of Zoology, Yale University.

Three basic physiological questions that may be asked about eyes are: (1) How are images formed? (2) Where does sensory excitation occur? (3) What sort of information is transmitted to the central nervous system? The following is a review of some recent work on these problems with respect to the structural and functional units (ommatidia) constituting the compound eyes of arthropods.

The basic elements making up single ommatidia are: a corneal lens, a crystalline cone, retinular cells, a rhabdom (a secretory product of the retinular cells?), optic nerve fibers, pigment cells (variable in distribution —usually two distal, several proximal), and a basal membrane. The sizes and relations between these elements vary greatly in different compound eyes.

The corneal lens and crystalline cone form the dioptric system of the ommatidium. The photosensitive structure is still unidentified, though the retinular cells, giving rise to the optic nerve fibers, must be stimulated in some way. The rhabdom has often been suggested in this rôle, primarily on the basis of the (often purplish) pigment seen in many rhabdoms. The fading of this pigment has been shown not to be proportional to exposure to light, however, and many forms have unpigmented rhabdoms. There are even some forms with good vision which lack rhabdoms. This theory therefore seems not well founded.

The photochemical mechanism involved in compound eyes is unknown. Kampa has recently extracted "euphausiopsin," a carotenoid protein complex, from the compound eyes of some pelagic shrimps, but nothing is known of its localization or reactions.

The distance from the crystalline cone to the rhabdom determines whether a given compound eye is a superposition (forms a single image) or an apposition (forms multiple images—one per ommatidium) eye. This distinction is not a hard and fast one, however, due to variations in struc

ture. Barlow has made a theoretical approach to the optical properties of ommatidia of various sorts.

Hanaoka has shown a marked increase in generator potential activity at the level of the rhabdom and retinulae in the superposition eyes of crayfish. This may well be significant in indicating the site of light energy absorption. Wiersma and Waterman have found only one active nerve fiber per ommatidium in the king crab (*Limulus*), though there are ten or more retinular cells per element. Does the ommatidium act as a unit?

Waterman has shown that single ommatidia will respond to light throughout a cone of some 70° from the optic axis of the ommatidium. There is thus a great overlap of optical fields and J. Muller's mosaic vision theory seems untenable. On the other hand, work with locusts shows that movements covering angles much smaller than the field of a single ommatidium (about 1°) are seen.

The eyes of many crabs show no continuous discharge in the fibers of the optic nerve studied. Responses to changes in light intensity and movement alone are recorded. The spiny lobster (*Panulirus*) also has additional fibers, however, which show continuous discharges.

Studies of behavior, especially using moving stripe patterns and the opto-kinetic nystagmus shown by stalk-eyed crustacea, are also contributing to understanding of these problems. The effects of polarized light, and the mechanism of polarized light sensitivity, are being studied.

M. S. G.

## CARDIOVASCULAR STUDY UNIT

February 20, 1956

CARDIOVASCULAR AND METABOLIC CONSEQUENCES OF RENAL FAILURE. By J. Russel Elkington, School of Medicine, University of Pennsylvania, Philadelphia.

In order to arrive at a more satisfactory method of management of acute renal failure, the study of the clinical entity of tubular necrosis with ensuing acute renal failure was undertaken. A series of 59 cases accumulated over an eight-year period was used. Of this group, 23 were treated in a conservative manner employing such measures as the conventional restriction of fluids to 700 to 1,000 cc. per day, absolute salt restriction during the oliguric phase, digitalis, and cation exchange resins. A 64 per cent mortality was experienced in this group. The remaining 36 cases in the series were treated by the use of a Skegg Leonard dialyzer. With this method of treatment mortality was only 36 per cent.

It is important to consider the patient as a closed system during the days preceding tubular functional regeneration and resultant diuresis. For this reason careful consideration of the patient's fluid needs and electrolyte status is essential. In the above study, fluid intake was restricted to approximately 650 cc. per day, or the mean difference between the insensible loss and the water of oxidation. In the group treated conservatively, fluid was withheld entirely for 24 or more hours lest the patient exhibit weight gain

in the face of anuria. It was concluded, however, that the treatment of choice and the one which provided the most facile control during the oliguric phase of the illness was to dialyze, using negative hydrostatic pressure. This allows for the correction of the manifestations of overhydration, acidosis, and hyperkalemia.

R. H. F.

BIOPHYSICS-ZOOLOGY SEMINAR

February 20, 1950

Effects of Radiation on the Egg Nucleus and Cytoplasm of the Wasp Habrobracon. By R. C. von Borstel, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

*Habrobracon* is a small wasp whose larvae live parasitically on the caterpillars of the Mediterranean flower moth. It has a life cycle comparable in length to that of *Drosophila*. Its eggs are airfoil shaped in longitudinal section and are some 600 microns long by 150 microns thick. The egg cytoplasm is mostly peripheral, in a layer about 10 microns thick, with the egg nucleus very near the surface on the convex side near the thicker end. The center of the egg is mostly yolk. Sex determination is of the haploid diploid type—males being haploid, females diploid. Females alone, therefore, are produced from fertilized eggs.

The present work is a study of some of the effects of ultraviolet, X-, and alpha particle radiation on various stages of the eggs of this form.

Mrs. Whiting has found eggs in the first prophase of meiosis to be some twenty times more resistant to X-rays than eggs in the first meiotic metaphase. She also produced androgenic males by irradiating females, then mating them (males howing the influence of the male genome only are so produced). The present work has produced more androgenic males via X-irradiation at first metaphase than at first prophase. The characteristics of the dose-response curve indicate that damage to cytoplasmically determined characters is also of importance here.

With ultraviolet radiation, nuclear irradiation has far greater effects (by several orders of magnitude) on hatchability (per cent of eggs hatching) than cytoplasmic irradiation. The nuclear dose response curve is in two parts, however, some 30% of the eggs being more resistant to u-v than the remainder. Perhaps nuclear movement deeper into the egg is a factor here.

Hatchability effects after nuclear u-v irradiation are partially reversible by exposure to light (photoreactivation). This does not occur with cytoplasmic irradiation.

Hatchability studies following alpha particle bombardment (particle range in tissue about forty microns) indicate that a single direct hit on the nucleus is enough to inactivate it. The inactivation cross-section is just a little less than the nuclear size. Cytoplasmic alpha irradiation is only weakly effective in reducing hatchability.

The u-v action spectra for both cytoplasmic and nuclear irradiation fairly closely parallel the absorption spectra of nucleic acids. Nucleic acids (prob-

ably as nucleoproteins) are thus likely the substances inactivated. There are differences in the mechanism of death following irradiation at different wave-lengths.

The embryological fates of the different parts of the egg are determined very early. Cytoplasmic irradiation produces defects in the appropriate areas in the adults hatching. Cytoplasmic influences on development are thus demonstrated.

M. S. G.

## POLIOMYELITIS CONFERENCE

February 22, 1956

RECENT ADVANCES IN DIAGNOSIS. By Dorothy Horstmann.

The diagnosis of poliomyelitis is particularly aided by the use of tissue culture and serological methods. The recognition of the two clinically different types of poliomyelitis, the biphasic and monophasic, is important diagnostically, both unto itself and in relation to the tissue culture and serological procedures which are to be performed. Thus, in the biphasic or childhood type of disease, a Minor Illness of transient sore throat and headache often precedes, by about five days, the Major Illness, which is characterized by sudden rise of temperature and central nervous system symptoms of stiff neck and paralysis. Inasmuch as the Major Illness is usually the presenting clinical entity, tissue culture studies should be performed on throat and particularly feces specimens rather than on blood, since the viremia disappears with the Minor Illness. The Major Illness is also characterized by the presence of complement-fixing and neutralizing antibodies of which only the former is useful for obtaining significant diagnostic titers. These methods and principles are also applicable for the diagnosis of the monophasic or adult type disease.

The tissue culture method may give positive results in 7 to 14 days and is especially useful for distinguishing the specific polio viral types (I, II, and III). While the results of the complement-fixing test may be obtained within 24 hours, the viral type specificity cannot be determined. The practical application of these methods in the differential diagnosis of encephalitis and the aseptic meningitides unrelated to polio, as well as in the diagnosis of atypical forms of polio is to be noted.

THE CURRENT STORY IN VACCINATION. By Francis L. Black and John R. Paul.

In evaluating the Salk poliomyelitis vaccine for its safety, a consideration of the consequences of sensitizing materials and infectious agents which might be present is required. An analysis of this sort indicates that the only major potential source of danger in the vaccine is the presence of the live polio virus itself—witness the Cutter Laboratories incident of a year ago. This problem is related, in large part, to the aggregation of virus particles which allows some of the particles having slow rates of formaldehyde in-

activation to escape destruction. The new regulations governing the manufacture and release of Salk vaccine have stressed the use of more adequate filtration methods to prevent particle aggregation. They require, in addition, that larger quantities of vaccine be tested and that all such tests prove consistently negative for the presence of live virus. While these more stringent precautions have aimed at completely safeguarding the vaccine, it should be pointed out that on theoretical grounds absolute safety is not obtainable. At present, therefore, the safety of the vaccine is primarily based upon the fact that only a very few sporadic and ill-defined cases of polio have been traced to its use from among the very large population vaccinated during the last year.

Although the Salk vaccine is considered safe to use (with the reservations noted above), the more immediate objective is the development of a vaccine from live attenuated virus. This aim evolves from a consideration of the natural history of the disease as well as from the relatively marked effectiveness and safety of such presently used attenuated vaccines as those for smallpox and yellow fever.

S. H.

PERIPHERAL VASCULAR DISEASE. By A. J. Barnet and J. B. E. Fraser. London and New York, Cambridge University Press, 1955. 219 pp. $9.50.

Doctors Barnet and Fraser of the Alfred Hospital Clinical Research Unit, Melbourne, Australia submitted this monograph for the Stawell Memorial Prize Essay of the British Medical Association for the year 1952 and were awarded the prize.

They believed a "fresh consideration" of peripheral vascular disease in order because of the great prevalence of this type of disease, the need for increasing accuracy in diagnosis of such lesions, and the importance at this time of assessing the more recent work that has been done with reference to etiology, pathogenesis, and treatment as this field has developed over the recent years.

The book is divided into seven chapters followed by a short summary. The first has to do with the history of knowledge of peripheral vascular disease. This is followed by an interesting chapter on the physiology of blood vessels, concerning both structural and dynamic characteristics. The chapter on diagnosis presents the special tests and apparatus which the authors consider have proved to be generally helpful clinically. The chapter on pathology is concisely presented and illustrated with a number of black and white photographs of gross and microscopic specimens. It dwells considerably on the pathogenesis of arteriosclerosis. The chapter on clinical features is presented from the standpoint of the syndromes caused by various diseases. These syndromes are then illustrated with case histories taken from the authors' clinical experience. The chapter on treatment includes a helpful discussion of the mechanism of action of drugs useful in this field. There is a final discussion as to prognosis of the different lesions. The text is supplemented with 31 figures and 29 plates, including a few in color.

Within the limits of 192 pages of text the authors do a remarkably good job in presenting the problems of peripheral vascular disease from the seven points of view indicated above, and as such I think this book will serve as an excellent introduction to the field. Moreover, it is unusually well documented so that though the text may be short and concise, the reader can easily pursue the subject further from the references, many of which are relatively recent.

With reference to the surgery of peripheral vascular disease, the book concerns itself mainly with brief discussion as to indications and occasionally as to relative worth of different procedures.

In short, this is a concise, well documented though brief, introduction to peripheral vascular disease based on a sound presentation of the physiology and pathology of blood vessels. It should prove useful to students, to general practitioners, and to those who would like a summary of recent trends in this field.

WILLIAM E. BLOOMER

PRIMARY ANATOMY. By H. A. Cates and J. V. Basmajian. Baltimore, The Williams & Wilkins Co., 1955. xii, 339 pp.

The book deals with anatomy systemically. It is easy to read and contains more than 440 line drawings which are exceptionally good. The material of the book was directed, "More than ever at students in Physiotherapy, Occupational Therapy, Physical and Health Education, Nursing and similar collegiate courses in Human Anatomy." It is probably adequate for students in these categories. However, the authors also state, "Although it (the book) is not directed primarily at medical students, they too, may find it useful because the essential principles and facts of Anatomy are emphasized here as in few other textbooks. In addition, there is much important information not readily available to them elsewhere." Some of this may be true, but I would not recommend it for medical students for the following reasons: (a) Many of the anatomical terms are too simplified; (b) The overly elementary manner in which certain structures are described, i.e., "The ascending, transverse, descending and pelvic parts of the colon form the sides of a 'picture-frame' which is deficient in the right lower corner. The contained 'picture' is the small intestine, etc.," "The hypophysis is a small gland about the size of a pea hanging by a stalk, the infundibulum . . . it rests, like an egg in its nest," etc., "If one could nibble a little of the mushroom that Alice carried in her right hand, one could perhaps become small enough to stand in the tympanic cavity," A retroperitoneal organ bulging into the peritoneal cavity with its covering of peritoneum is compared with, "the hump produced by a small boy hiding under a carpet"; (c) Questionable statements, i.e., "The ovum compensates for the scarcity of its numbers by its size"; (d) Erroneous descriptions, i.e., "From the fibrous annulus, ring or cuff arise five ocular muscles and one muscle which raises the upper lid," "Mesenteries: Other organs—requiring greater mobility—progress much farther and 'break away' from the posterior (body) wall. However, this 'breaking away' cannot be complete because the organs must still receive their many vessels and nerves. These vessels and nerves pass to the 'free' organs between the two layers of peritoneum which the organ has 'pulled off' from the posterior abdominal wall."

E. S. ORELLS

IONIC INTERPRETATION OF DRUG ACTION IN CHEMOTHERAPEUTIC RESEARCH. By Alexander V. Tolstoouhov. New York, Chemical Publishing Co., Inc., 1955. 270 pp. $10.00.

The author of this monograph holds that the most productive approach to chemotherapeutic research is to search for relationships between the biological activities of drugs and the physico chemical characteristics of the drugs and of the tissues (or microorganisms) on which they act. This would not only explain the actions of drugs, but it would also simplify the design of new drugs.

His own approach is presented in this highly personal monograph. At the outset he draws a parallel between selective staining and selective drug action, both of which depend on four variables: The dissociation constant

of the chemical, the isoelectric points of the ampholytes of the tissues or microorganisms, the pH of the solution, and the solubility of the drug-tissue complex. The activities of local anesthetics, hypnotics, sulfonamides, and other compounds are related to their basic dissociation constants which allow them to react with nerve cells and bacteria because at the pH of tissue fluids, the drug and its target carry opposite charges. The isoelectric points of nerve cells (2.5) and bacteria (0.5 for gram positive organisms, 2.5 for gram negative) are close, and the dissociaton constants of hypnotics and sulfonamides are close; these facts may explain the reason sulfonamides affect the nervous system. Drugs and toxins can be detoxified by administering substances of opposite charge that combine with the toxic agent. Thus ". . . we may say that the success of treatment of arthritis with p-aminobenzoic acid and acetylsalicylic acid depends on the ability of these acids to neutralize some of the poisonous substances which are evidently present in cases of arthritis." The alleged treatment of toxemia of pregnancy, barbiturate coma, and rheumatoid arthritis by ascorbic acid, and of hepatic coma by glutamic acid rest on the same mechanism.

The author's original idea, while not new, is interesting, but he makes too much of inorganic and *in vitro* experiments, which are gingerly extrapolated to living systems, and of isolated and unsubstantiated clinical reports. Aside from this shortcoming the book is discursive, repetitious, and prolix, windy with long quotes. But there are enough croppers to startle the reader into wakefulness, like "The necessity of large doses (of sulfamlamide) continuously administered for a long time immediately excludes enzymelike action . . ."

J. P. G.

THE HOUSE PHYSICIANS HANDBOOK. By C. Allan Birch. Edinburgh and London, E. and S. Livingstone Ltd., 1955; Baltimore, Williams and Wilkins Company. 160 pp. $3.00.

This little handbook was written for house officers working in British Regional Hospitals. It contains a general discussion of the resident's duties and responsibilities and some of the legal and ethical problems he is likely to encounter, a description of common clinical and laboratory procedures, and a section on therapy. The scope of the material covered is astounding and includes such varied and unexpected subjects as a working French, German, and Italian vocabulary, the technique of embalming, the correct form of emergency baptism, and an ingenious method for removing a fixed wedding ring! Due to the limitations of space the discussions are of necessity very brief and follow one another in rapid, disorderly succession. Although the book makes interesting reading and, on the whole, provides sound advice, it is doubtful that it will satisfy the sophisticated house officer who has access to even a very meager working library.

GERALD KLATSKIN

PREVENTIVE MEDICINE IN WORLD WAR II. Vol. II, ENVIRONMENTAL HYGIENE. John Boyd Coates, Jr., Editor-in-Chief. Washington, D. C., U.S. Government Printing Office, 1955. vii, 404 pp. $3.50.

This volume is the first of a series to be published recording the accomplishments of various divisions of the Preventive Medicine Service of the U.S. Army during World War II. The whole story of these accomplishments, which took a variety of forms and were carried out in combat on many fronts, is a record of how preventive medicine grew mightily during the past generation and particularly during the twenty two years between World War I and World War II. It will stand as a monument to the late General James S. Simmons, who directed the program from the Office of the Surgeon General of the Army. This particular volume deals specifically with that portion of the program which comes under the broad term of "Environmental Hygiene."

Since early antiquity, wise military leaders have recognized the importance of maintaining a high standard of cleanliness among their troops. The practical application of this ideal was improved when new knowledge based on microbiology and epidemiology showed that the broad term of "cleanliness" could be applied specifically to the prevention of disease through sanitation of the environment. The extraordinary degree to which these practical applications not only kept pace with the rapid development of knowledge, but were used in the field of military medicine, was one of the most remarkable achievements in World War II and one which resulted in the prevention of disease to a larger extent than it is perhaps possible to measure or even imagine.

The story is told here largely by two Army Officers of considerable experience: Col. T. F. Whayne of the Medical Corps, and Col. W. A. Hardenberg of the Sanitary Corps. The chapters cover the military aspects of: food management—as carried out in the U.S. Army in World War II; housing; water purification; waste disposal; the control of insects; foreign quarantine; and the practice of preventive medicine in ports of embarkation and for persons in transit. Into these fields many new principles and new methods have found their way to keep pace with the demands which our modern civilization makes for controlling the environment for purposes of health. Perhaps none of these fields has undergone as sudden a transformation as that of the control of insect vectors of disease such as mosquitoes, lice, and sand flies. Outstanding therefore, among special features in the preventive medicine program in World War II was the initiation of research which led to the discovery, development and application of many new agents for the control of insect borne diseases which have often harried troops operating in the field, and certainly were a great menace both in the Mediterranean area and the South Pacific. The story is told here of the more effective methods for the application of one of these insecticides, namely DDT. This provided a powerful weapon where it was badly needed, and one which, since 1945, has been used to rid mankind of a host of former plagues, including malaria. In this day and age we assume, perhaps too blandly, that our water supplies are to be pure, our houses are to be heated,

and sewage is to be properly removed. Most soldiers have been brought up in an environment where these standards are the rule. The degree to which such standards can suddenly be furnished and maintained within large hurriedly constructed Army camps in wartime, and provided for the soldier in the field, and for the soldier in combat, was the monumental task described in this volume. It is a record of accomplishment in which medical officers, engineers, research workers, and a host of other planners and builders took part. In retrospect their accomplishments during World War II may not have been as spectacular as the achievements, for instance, in plastic surgery and microbiology in restoring man to health,—but the end results are probably as great.

JOHN R. PAUL

DONOVANOSIS (GRANULOMA INGUINALE, GRANULOMA VENEREUM). By R. V. Rajam and P. N. Rangiah. World Health Organization Monograph No. 24. Geneva, World Health Organization, 1954 (distributed by Columbia University Press, New York). 46 pp. $1.50.

This volume is a clear, systematic, critical presentation of the subject by two authors with extensive clinical experience and succeeds admirably in providing maximal information in minimal space. The illustrations are varied and informative.

Donovanosis is proposed as the preferred name to replace the many synonyms which are imprecise or are misleading as to site of election or are readily confused with lymphogranuloma. The point seems well chosen; the name appropriate and meaningful.

Clinical variation is stressed, for failure to recognize, and accordingly treat, may lead to great distress and eventually to deformity and mutilation. About 90% of the lesions are genital with extension to neighboring areas, and a prevalent notion of primary inguinal involvement should be discarded. Extragenital lesions are nearly always oral and almost invariably follow long standing genital granulomata; in a few cases Donovanosis is confined to the anal and perianal region. Deep-seated lesions involving the vagina and cervix may occur in long-standing infections, and mention is made of involvement of the liver and bladder, and also osteomyelitis. Yet, in the usual case constitutional manifestations, including fever, are absent, neither preceding nor accompanying the disease.

Transmission is primarily venereal, but there are many curious failures to transmit even under what appear to be the most propitious circumstances. The authors reject the idea that this points to extra-venereal acquisition and instead suggest factors of low transmissibility and individual resistance. These factors would also account for the low incidence of Donovanosis in the population at large, for, given a free field and no favor, it should be somewhere near as common as syphilis and gonorrhea, and yet it is not.

Diagnosis is made by the identification of Donovan organisms in films prepared from a fragment of granulation tissue. Typically, *D. granulomatis* appears as a rod-shaped body, encapsulated or not, often occurring intra-

cellularly in mononuclear or more rarely, polynuclear cells, and then often situated within "cysts." In such cases recognition should offer no great difficulty, but in early lesions where, it is said, the organism may be atypical and exclusively extracellular, morphological identification would seem to present problems. In such cases, the complement fixation and skin tests are stated to be of little assistance, and their chief utility is in clinically suspicious cases as a confirmatory negative finding when organisms also cannot be found.

Satisfactory treatment has become enormously facilitated with the introduction of four antibiotics which are effective. Failure with any one of these, presumably from resistance, may be easily coped with. It is pointed out by the authors that with the low incidence rate of Donovanosis and its poor transmissibility, organized public health measures concentrating on early diagnosis and effective therapy should exert very effective control. Medicine has given its answer; the remaining problem is the whereabouts of the wherewithal.

DAVID WEINMAN

THE MEDICAL CARE OF THE AGED AND CHRONICALLY ILL. By Freddy Homburger. Boston and Toronto, Little Brown and Company, 1955. 253 pp. $5.75.

This volume is intended for practitioners, medical students, nurses, and "intelligent laymen" who face the many problems which are created by chronic illness and by the varying needs of aged and incapacitated persons. Its emphasis is on chronic illness among older persons rather than on the many complex problems of younger people. It should be useful to hospital administrators and their staffs, to social workers, and to those engaged in mental health and public health work. Raoul Dufy's artistic illustrations are unique additions from his personal experience as a patient in the hospital.

The nine chapters deal with the rôle of the physician in the management of chronic illness, of osteoporosis, of arthritis, of malnutrition, and with the management of advanced cancer, of hemiplegia, of paraplegia, and of complications (urinary, gastrointestinal, central nervous system; also bed and pressure sores), and with nursing problems, including care and apparatus.

Practical guidance is offered for the handling of special situations given "little attention in the curriculums of medical schools and which present irksome problems for those who have to meet them and cope with them in day-to-day practice." It is suggested that an understanding attitude on the part of those in charge may mean the difference between a hopeless and useless existence and rehabilitation to a useful and far more tolerable life. There are challenges here for the family doctor—"the one who should be most competent and alert in these matters," and for those responsible for beds ("constructed for the comfort of the attendants rather than for that of the patient"), bedclothes ("designed for nurses' convenience rather than for the patients' comfort"), and the bed pan ("the worst of all hospital torture

tools"). There are numerous useful references following each chapter and a practical index.

This enlightening book by a physician experienced in research and teaching is a useful working text. With no claim to completeness, those subjects are emphasized wherein progress has been made recently, "enabling medicine to render effective relief, if not to provide a cure."

IRA HISCOCK

INTERNATIONAL ENCYCLOPEDIA OF UNIFIED SCIENCE. Otto Neurath, Rudolf Carnap, and Charles W. Morris, Eds. Vol. I, Parts I and II. Chicago, The University of Chicago Press, 1955. ix, 760 pp. Each part, $6; set, $11.

The publication of this authoritative series of papers in two manageable volumes is a fortunate event. The reading public is already familiar with one or the other of the individual parts making up these books, and to praise them now would be anachronistic. They flow from a philosophical view which originated in the so-called Vienna Circle, of which several of the authors were original members. The contents are:

Part I: Encyclopedia and Unified Science
- Otto Neurath: Unified Science as Encyclopedic Integration, 1-27
- Niels Bohr: Analysis and Synthesis in Science, 28
- John Dewey: Unity of Science as a Social Problem, 29-38
- Bertrand Russell: On the Importance of Logical Form, 39-41
- Rudolf Carnap: Logical Foundations of the Unity of Science, 42-62
- Charles W. Morris: Scientific Empiricism, 63-75
- Charles W. Morris: Foundations of the Theory of Signs, 79-137
- Rudolf Carnap: Foundations of Logic and Mathematics, 143-213
- Leonard Bloomfield: Linguistic Aspects of Science, 218-277
- Victor F. Lenzen: Procedures of Empirical Science, 281-339

Part II: Ernest Nagel: Principles of the Theory of Probability, 341-422
- Philipp Frank: Foundations of Physics, 423-504
- E. Finlay-Freundlich: Cosmology, 505-565
- Felix Mainx: Foundations of Biology, 567-654
- Egon Brunswik: The Conceptual Framework of Psychology, 655-760

The quality of the writing is attested to by the fame of these authors. If a criticism were to be levelled at the work, it would concern the over-all title of the book. It is international in a measure because of the origin of the contributors, hardly because of their present locations. It is not an encyclopedia in the usual sense of the word; it unifies science within the framework of logical positivism.

HENRY MARGENAU

CHILD BEHAVIOR. By Frances L. Ilg and Louise Bates Ames. New York, Harper and Brothers, 1955. xi, 304 pp. $3.95.

This book, in the words of Dr. Arnold Gesell who wrote the foreword, "incorporates and adapts" material from the syndicated newspaper column which has been written by the authors since 1951. Its apparent intent is to provide for parents a helpful guide to child rearing by dealing more specifically with problems than has been done in earlier publications.

The "ages and stages" philosophy, while important, is again impressively overemphasized. The authors make some statements about which there is general agreement, for example, that individuality, environment, and developmental phases are all important. The superficiality of their consideration of environmental influences is disappointing, as is the absence of any real evidence that they believe the feelings which flow between parent and child influence development in many important ways.

Some of their descriptions of behavior are graphic and their anecdotal material is entertaining. However, their advice in regard to specific situations is so mechanistic as to make no allowance for the individuality of a child and parent. They present no basis for the parents' thinking about the meaning of behavior. They often convey the attitude that all the parent really needs is the hardiness to survive until a smoother "phase" presents itself.

To this reviewer their basic philosophy about development is not only a limited but a depressing one. The inevitability, for example, of having one's development and behavior "largely determined" by having been born a mesomorph or an ectomorph, or to feel that there is nothing to do in a difficult period except to wait for the next stage is far from helpful.

SALLY PROVENCE

THE THYROID. Brookhaven Symposium in Biology No. 7. A. Edelmann, H. J. Curtis, and M. E. Koshland, Eds. Washington, D. C., Office of Technical Services, Department of Commerce, 1955. vii, 271 pp.

This volume contains transcriptions of the fifteen papers presented at the symposium on the thyroid at Brookhaven on June 9 to June 11, 1954 together with summaries of the discussion which followed the presentations. The authors are uniformly and eminently qualified and the material ranged from phylogenetic considerations through biochemical and physiological discussions to clinical applications. Each paper contains a massive review of the literature together with presentation of the author's own observations. Some of the literature reviews are very complete including over 200 references apiece. Although most of the material presented in these discussions has appeared elsewhere there is great convenience in having it all gathered in a single volume. The only serious criticism arises from the fact that the discussion has not been edited as rigorously as one might desire. As a result some of the statements are garbled and even, in a few instances, incomprehensible. In spite of a few such lapses the book is certainly worth the careful study of any biologist who is seriously interested in the metabolism and diseases of the thyroid gland.

PHILIP K. BONDY

## ADDITIONAL BOOKS RECEIVED

Immunology and Serology. By Philip L. Carpenter. Philadelphia, W. B. Saunders Company, 1956. vii, 351 pp. $6.50.

A Shorter Version of the Second Edition of THE VERTEBRATE BODY. By Alfred S. Romer. Philadelphia, W. B. Saunders Company, 1956. viii, 485 pp. $5.50.

The Microbe's Contribution to Biology. By A. J. Kluyver and C. B. van Niel. Cambridge, Mass., Harvard University Press, 1956. 182 pp. $4.00.

Endocrinology Society Memoirs, No. 4, *Comparative Physiology of Reproduction* I. Chester Jones and P. Eckstein, Eds. New York, Cambridge University Press, 1956. x, 253 pp. $8.50.

Endocrinology Society Memoirs, No. 5, *Hormonal Control of Water . . . etc.* I. Chester Jones and P. Eckstein, Eds. New York, Cambridge University Press, 1956. ix, 124 pp. $4.75.

The Preservation of Natural History Specimens, Volume I, Invertebrates. New York, Philosophical Library, Inc., 1955. xiii, 205 pp. $10.00.

Nellie Landblom's Copybook for Beginners in Research Work. By Nellie Thompson Landblom. Multigraph Service Bureau, Colorado, 1955. 118 pp. $2.95.

Aspects of Synthesis and Order in Growth. Dorthea Rudnick, Ed. New Jersey, Princeton University Press, 1955. vii, 274 pp. $6.00.

The Complete Medical Guide. By Benjamin F. Miller. New York, Simon and Schuster, 1956. xii, 913 pp. $4.95.

Experimental Psychology. B. A. Farrell, Ed. A Series of Broadcast Talks by A. J. Watson, Harry Kay, J. A. Deutsch, B. A. Farrell, Michael Argyle, R. C. Oldfield. New York, Philosophical Library, Inc., 1955. xi, 66 pp. $2.75.

The Physiology of Insect Metamorphosis. By V. B. Wigglesworth. New York, Cambridge University Press, 1954. viii, 152 pp. $2.50.

Introduction to Virology. By Gilbert Dalldorf. Springfield, Illinois, Charles C Thomas, 1955. ix, 102 pp. $3.50.

An Introduction to Human Anatomy. By Clyde Marshall and Edgar L. Lazier. Philadelphia, London, W. B. Saunders Company, 1955. xiii, 420 pp. $4.50.

Hypnosis and Its Therapeutic Applications. By Roy M. Dorcus, Ed. New York, McGraw-Hill, 1956. x, 294 pp. $7.50.

The Rôle of Algae and Plankton in Medicine. By Morton Schwimmer. New York, Grune & Stratton, Inc., 1955. x, 85 pp. $3.75.

Tuberculosis in the Army of the United States in World War II: An Epidemiological Study with an Evaluation of X-ray Screening. By Esmond R. Long and Seymour Jablon. Washington, D. C., U.S. Government Printing Office, 1955. vii, 88 pp. $1.50.

## ADDITIONAL BOOKS RECEIVED

A Follow-Up Study of World War II Prisoners of War. By Bernard M. Cohen and Maurice Z. Cooper. Washington, D. C., U.S. Government Printing Office, 1954. vii, 81 pp. $1.50.

Atomic Energy Research at Harwell. By K. E. B. Jay. New York, Philosophical Library, Inc., 1955. xii, 144 pp. $4.75.

Old People in a Modern Australian Community. By Bertram Hutchinson. New York, Cambridge University Press, 1954. xii, 180 pp. $4.75.

Autoradiography in Biology and Medicine. By George A. Boyd. New York, Academic Press Inc., 1955. xiii, 399 pp. $8.80.

Food Poisoning. By G. M. Dack. Chicago, Illinois, The University of Chicago Press, 1956. viii, 251 pp. $6.00.

Textbook of Occupational Therapy. By Eamon N. M. O'Sullivan. New York, Philosophical Library, Inc., 1955. x, 319 pp. $10.00.

The Physiology of Diapause in Arthropods. Cambridge Monographs in Experimental Biology 4. By A. D. Lees. New York, Cambridge University Press, 1955. x, 150 pp. $2.50.

Expression of the Emotions in Man and Animals. By Charles Darwin. Introduction by Margaret Mead. New York, Philosophical Library, Inc., 1955. xi, 372 pp. $6.00.

Advances in Virus Research, Volume III. Kenneth M. Smith and Max A. Lauffer, Eds. New York, Academic Press Inc., Publishers, 1955. ix, 399 pp. $8.00.

Psychiatry for the Family Physician. By C. Knight Aldrich. New York, McGraw-Hill, 1955. vii, 276 pp. $5.75.

The Biological Effects of Tobacco. Ernest L. Wynder, Ed. Boston, Little, Brown & Co., 1955. xiii, 213 pp. $4.50.

Man in a Cold Environment. By Alan C. Burton and Otto G. Edholm. Baltimore, William & Wilkins Co., 1955. xiv, 273 pp. $6.75.

Management of Addictions. By Edward Podolsky. New York, Philosophical Library Inc., 1955. xvii, 413 pp. $7.50.

Vitamins in Theory and Practice. 4th ed. By L. J. Harris. New York, Cambridge University Press, 1955. xxii, 300 pp. $6.50.

One in Six: An Outline of the Cancer Problem. By I. Hieger. London, Allan Wingate Ltd., 1955. 80 pp. 12s/6d.

The Liver and Cancer. By Kasper Blond. Bristol, England, John Wright & Sons Ltd., 1955. xii, 220 pp. $6.50.

Some Physiological Aspects and Consequences of Parasitism. William H. Cole, Ed. New Brunswick, New Jersey, Rutgers University Press, 1955. xi, 90 pp. $2.00.

The Medical Significance of Anxiety. By Richard L. Jenkins. The Biological Sciences Foundation, Ltd., Washington, D. C., 1955. 46 pp. $1.00.

Adrenal Cortex: Transactions of the Fifth Conference November 4, 5, and 6, 1953, Elaine P. Ralli, Ed. New York, The Josiah Macy, Jr. Foundation, 1954. 187 pp. $3.75.

Problems of Consciousness: Transactions of the Fifth Conference March 22, 23 and 24, 1954, Harold A. Abramson, Ed. New York, The Josiah Macy, Jr. Foundation, 1955. 180 pp. $3.50.

Group Processes: Transactions of the First Conference September 26, 27, 28, 29, and 30, 1954. Bertram Schaffner, Ed. New York, Josiah Macy, Jr. Foundation, 1955. 334 pp. $5.50.

Cybernetics: Circular Causal and Feedback Mechanisms in Biological and Social Systems. Transactions of the Tenth Conference, April 22, 23 and 24, 1953. Heinz Von Foerster, Ed. Princeton, New Jersey, New York, The Josiah Macy, Jr. Foundation, 1955. 100 pp. $2.75.

Shock and Circulatory Homeostasis: Transactions of the Fourth Conference, December 6, 7, and 8, 1954. Harold D. Green, M.D., Ed. New York, Josiah Macy, Jr. Foundation, 1955. 291 pp. $5.00.

New Age Determinations by the Lead Method. Roy Waldo Miner, Ed. Annals of the New York Academy of Sciences, Vol. 60, Art. 3, pages 509-520. New York, 1954. $.50.

Instrumentation. Roy Waldo Miner, Ed. Annals of the New York Academy of Sciences, Vol. 60, Art. 6, pages 807-964. New York, 1955. $3.00.

Recent Advances in the Study of the Structure, Composition, and Growth of Mineralized Tissues. Roy Waldo Miner, Ed. Annals of the New York Academy of Sciences, Vol. 60, Art. 5, pages 541-806. New York, 1955. $4.00.

A General Theory of Association. Roy Waldo Miner. Ed. Annals of the New York Academy of Sciences, Vol. 60, Art. 4, pages 521-540. New York, 1955. $.75.

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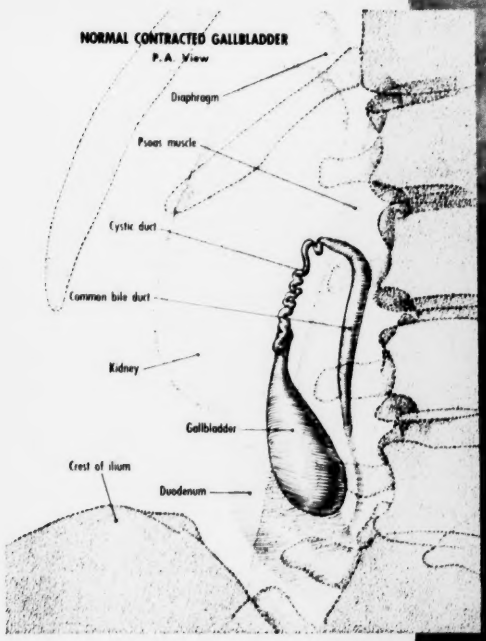

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Buckstein, Jacob: The Digestive Tract in Roentgenology. Philadelphia, J. B. Lippincott Co., 2nd ed., 1953, vol. 2, p. 1003.

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